Uniwersytet Medyczny w Białymstoku

DZIEDZINA: NAUKI MEDYCZNE I NAUKI O ZDROWIU

DYSCYPLINA: NAUKI MEDYCZNE

ROZPRAWA DOKTORSKA

Ekspresja transporterów substratów energetycznych u pacjentek z zaawansowanym surowiczym rakiem jajnika

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Rozprawa doktorska została zrealizowana w ramach kształcenia w Szkole Doktorskiej UMB

Białystok, 2023

Medical University of Bialystok

FIELD OF SCIENCE: MEDICINE

SCIENTIFIC DISCIPLINE: MEDICINE

DOCTORAL THESIS

Gene expression in energy substrate transporters in High-Grade Ovarian Cancer

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The doctoral thesis was carried out as a part of education at the Doctoral School of MUB

Białystok, 2023

Pragnę podziękować każdemu,

kto pokazał mi prawdziwe piękno nauki i przyczynił się do powstania tej pracy.

Białystok, 2023

Praca została sfinansowana przez:

Uniwersytet Medyczny w Białymstoku w ramach zadań badawczych o numerze:

SUB/1/DN/22/007/1118.

Białystok, 2023

Spis treści

Białystok, 2023

Białystok, 2023

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Kursy zawodowe

1. „Diagnostyka Prenatalna” 21-22.01.2022 r.
2. „Ultrasonografia w położnictwie i perinatologii 05-06.11.2021 r.
3. „USG sutków z uwzględnieniem elastografii dla średniozaawansowanych.” Kurs praktyczny 28-30.05.2021 r.
4. I PARP FORUM 2019 – Rola i znaczenie PARPi w raku jajnika 22–23.11.2019
5. „Anatomical aspects of the cytoreductive ovarian cancer surgery for young gynecologist oncologists” 28-29.02.2020r Wrocław
6. „Wady płodu – diagnostyka prenatalna na podstawie badań USG” 29.04-25.05.2022 r.
7. „Ultrasonografia w Perinatologii – wybrane problemy” 29.04-25.05.2022 r.
8. „Podstawy diagnostyki USG sutków. Kurs praktyczny" 17-19.03.2023 r.
9. „USG w położnictwie – poziom średniozaawansowany” 28-29.10.2022 r.
10. „USG w położnictwie - poziom zaawansowany” 25-26.11.2022 r.
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3. Studenckie Koło Naukowe przy Klinice Laryngologii, Uniwersytet Medyczny w Białystoku – 2018 r.
4. Studenckie Koło Naukowe przy Klinice Pediatrii, Reumatologii, Immunologii i Chorób Metabolicznych, Uniwersytet Medyczny w Białystoku - 2017 r.
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Białystok, 2023

Zestawienie publikacji

Rodzaj publikacji	Liczba	IF wg JCE na rok publikacji	Punktacja MEiN
Prace przeglądowe włączone do rozprawy	1	4,927	140
Prace oryginalne włączone do rozprawy	1	5,6	140
Prace oryginalne niewłączone do rozprawy	2	4,6	210
Opisy przypadków i prace przeglądowe niewłączone do rozprawy	3	2,046	145
Razem	**7**	**17,173**	**635 pkt.**

Białystok, 2023

Rozprawa doktorska

Ekspresja transporterów substratów energetycznych u pacjentek z zaawansowanym surowiczym rakiem jajnika

Autor: Marta Baczewska

Promotor pracy: prof. dr hab. Paweł Knapp

1. Artykuły stanowiące cykl prac włączonych do rozprawy doktorskiej

Nazwa czasopisma	**Tytuł artykułu**	**IF wg JCE na rok publikacji**	**Punktacja MEiN**	**Data publikacji**	**Rodzaj publikacji**
Molecules	Obesity and Energy Substrate Transporters in Ovarian Cancer-Review	4,927	140	16.03.2021	Praca przeglądowa
International Journal of Molecular Sciences	Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications	5,6	140	11.08.2022	Praca oryginalna

Białystok, 2023

2. Wykaz stosowanych skrótów i oznaczeń

ADNEX model *(ang. Assessment of Different NEoplasias in the adneXa)*

AATs (*ang. amino acid transporters*) – transportery aminokwasów

ASCT2 (ang. Alanine, Serine, Cysteine Transporter 2) – transporter alaniny, seryny, cysteiny typu 2

β-HAD (ang. 3-hydroxyacyl-CoA dehydrogenase) - dehydrogenaza hydroksyacylo-CoA

COX4/1 *(ang. Cytochrome c oxidase subunit 4 isoform 1)* – oksydaza cytochromu C podjednostka 4, izoforma 1

CPT1 (*ang. carnitine palmitoyltransferase I)* -palmitoilotransferaza karnitynowa 1

Ca125 - antygen nowotworowy 125

ESGO (*ang. The European Society of Gynaecological Oncology*) - Europejskie Towarzystwo Onkologii Ginekologicznej

FASN (*ang. fatty acid synthase*)– gen syntazy kwasów tłuszczowych

FABP (*ang. fatty acid binding protein)* – białko wiążące kwasy tłuszczowe

FABPpm (*ang. plasma membrane fatty acid binding protein*) - błonowe białko wiążące kwasy tłuszczowe

FATPs (*ang. fatty acid transport proteins*) - białka transportujące kwasy tłuszczowe

FAT/CD36*/SR-B2* (*ang. fatty acid translocase*) - translokaza kwasów tłuszczowych

FIGO (*fr. Fédération internationale de gynécologie et d'obstétrique*) - Międzynarodowa Federacja Ginekologii i Położnictwa, Międzynarodowa Federacja Ginekologów i Położników

GLUT (*ang. glucose transporter*) - białkowy transporter glukozy

HGSC *(ang. High-grade Serous Ovarian Cancer*) – rak surowiczy jajnika o wysokim stopniu złośliwości

inhibitor PARP *(ang. poly ADP ribose polymerase inhibitor)* - inhibitor polimerazy poli(ADP-rybozy)

HRT – *(ang. hormonal replacement therapy)* – hormonalna terapia zastępcza

IOTA (*ang. International Ovarian Tumor Analysis)* - Grupa Analizy Guzów Jajnika

LPL (*ang. lipoprotein lipase)* – lipaza lipoproteinowa

LAT (*ang. large neutral amino acid transporter)* – neutralny transporter dokomórkowy aminokwasów

MCT *(ang. monocarboxylate transporter)* - transporter monokarbolsylowy

NCCN *(ang. National Comprehensive Cancer Network)*

OC (ang. ovarian cancer) – rak jajnika

PGC-1α *(ang. peroxisome proliferator-activated receptor γ coactivator 1α*) - koaktywator 1α receptora γ aktywowanego przez proliferatory peroksysomów

ROMA *(ang. risk of ovarian malignancy algorithm*) - algorytm oceny ryzyka obecności raka nabłonkowego jajnika

ROS (ang. reactive oxygen species) – reaktywne formy tlenu

VEGF (*ang. vascular endothelial growth factor)* - czynnik wzrostu śródbłonka naczyniowego

SNAT1 (*ang. sodium-coupled neutral amino acid transporter 1)* - neutralny transporter aminokwasów sprzężony z sodem typu 1

TCGA *(ang. The Cancer Genome Atlas)* - atlas genomu nowotworów

TTP (*ang. time to progression)* – czas do progresji

TFAM (*ang. mitochondrial transcription factor A)* – mitochondrialny czynnik transkrypcyjny

TSH *(ang. thyroid-stimulating hormone)* - Tyreotropina, hormon tyreotropowy

OS *(ang. overall survival)* – przeżycie całkowite

3. Wstęp

3.1 Epidemiologia raka jajnika

Rak jajnika (OC) jest istotnym klinicznie i społecznie nowotworem złośliwym. Jest trzecim co do czestości nowotworem ginekologicznym i stanowi wiodącą przyczynę zgonów kobiet z przyczyn nowotworowych. Średni odsetek 5-letnich przeżyć wynosi około 50%. Najniższy jest w zaawansowanych stadiach i wynosi 31%, natomiast w przypadku choroby ograniczonej do jajnika sięga 93%. Na przestrzeni ostatnich pięćdziesięciu lat obserwuje się stopniowy spadek zachorowalności i śmiertelności, co może być związane ze wzrostem dostępności doustnej antykoncepcji oraz poprawą dostępności do nowoczesnych form leczenia [1] – [3]. Rak jajnika coraz częściej nazywany jest chorobą przewlekłą, która stanowi wyzwanie diagnostyczne, terapeutyczne i ekonomiczne. Opieka nad pacjentką wymaga zaangażowania zespołu wielodyscyplinarnego, złożonego z doświadczonych chirurgów, onkologów klinicznych, anestezjologów, fizjoterapeutów i psychologów. Choroba dotyka najczęściej kobiet pomiędzy 50 a 70 rokiem życia, lecz może wystąpić w każdym wieku [4].

3.2 Rodzaje raka jajnika

Rak jajnika jest niejednorodną grupą nowotworów, które różnią się między sobą histologicznie i molekularnie. Wyróżniamy nowotwory wywodzące się z tkanki nabłonkowej, łącznej i germinalnej. Najczęstsze z nowotworów złośliwych są te wywodzące się z tkanki nabłonkowej. Wśród raków wyróżniamy raka surowiczego o wysokim stopniu złośliwości (ang. HGSOC High-Grade Ovarian Cancer), który jest najczęstszy i stanowi 70%. Pozostałe typy to: rak endometrioidalny - 10%, rak jasnokomókowy - 10%, rak śluzowy - 3% oraz rak surowiczy o niskiej złośliwości, który stanowi mniej niż 5 % [5].

Z uwagi na częstość występowania i wciąż niewystarczająco skuteczne metody terapii oraz wczesnej diagnostyki niniejsza praca poświęcona jest podtypowi histologicznemu jakim jest HGSOC.

3.3 Rozpoznanie raka jajnika

Wczesny rak jajnika nie daje objawów klinicznych, a pacjentki nierzadko zgłaszają się po pomoc lekarską w momencie pojawienia się wodobrzusza, duszności bądź uczucia pełności

jamy brzusznej. W praktyce ginekologicznej w trakcie badania ultrasonograficznego transwaginalnego oceniany jest narząd rodny. Dzięki grupie IOTA (ang. International Ovarian Tumor Analysis) i systemowi zasad i definicji, który służy do jednolitej oceny zmian przydatków można w sposób systematyczny klasyfikować guzy i kwalifikować chore do leczenia wysokospecjalistycznego w ośrodkach dedykowanych operacjom raka jajnika np. akredytowane centra ESGO (ang. The European Society of Gynaecological Oncology) [6]. Udowodniono przewagę wysokiej jakości badania ultrasonograficznego i modelu ADNEX (ang. Assessment of Different Neoplasias in the adnexa) nad algorytmem ROMA (ang. Risk of ovarian malignancy algorithm), który nie ma zastosowania w wykrywaniu wczesnych raków [7][8]. W celu określenia stopnia zaawansowania klinicznego nowotworu stosuje się klasyfikację wg FIGO (fr. Fédération internationale de gynécologie et d'obstétrique) [9]. Pomocnicze mogą być badania obrazowe tj. tomografia komputerowa z kontrastem lub/i rezonans magnetyczny.

Pomimo postępu medycyny brakuje testu przesiewowego umożliwiającego szybką diagnozę, a metody wczesnego wykrywania guzów jajnika są niedoskonałe. Naukowcy poszukują zatem nowych markerów biochemicznych, które mogłyby stanowić proste, tanie i dostępne narzędzie diagnostyczne.

3.4 Leczenie raka jajnika

Leczenie powinno być zindywidualizowane, ponieważ grupa chorych jest niejednorodna pod względem stanu klinicznego, wrażliwości na chemioterapię czy biologii guza [10]. Postępowanie opiera się na aktualnych wytycznych tworzonych przez międzynarodowe towarzystwa naukowe skupiające zarówno ginekologów jak i onkologów klinicznych. Podstawę wytycznych europejskich dotyczących postępowania, diagnostyki, leczenia i profilaktyki można znaleźć w zbiorowej pracy dwóch towarzystw: Europejskiego Towarzystwa Onkologii Medycznej oraz Europejskiego Towarzystwa Onkologii Ginekologicznej - konsensus ESMO-ESGO zawarty w 2018 roku w Mediolanie. Zaktualizowaną wersję wytycznych amerykańskich przedstawiło w 2022 r. NCCN (ang. National Comprehensive Cancer Network), które jest siecią 32 wiodących ośrodków onkologicznych w Stanach Zjednoczonych [11]. Leczenie najczęściej polega na pierwotnej operacji cytoredukcyjnej, uzupełniającej chemioterapii i leczeniu biologicznym. Przełomowe było wprowadzenie do codziennej praktyki pochodnych platyny - karboplatyny, taksanów tj. paklitaksel, bewacyzumabu (rekombinowane przeciwciało monoklonalne wiążące czynniki wzrostu

śródbłonka naczyniowego VEGF) oraz inhibitorów PARP (polimeraz poli (ADP-rybozy) tj. olaparib czy niraparib które dają szansę na wydłużenie czasu do progresji (ang. time to progression - TTP) lub nawet czasu przeżycia całkowitego (ang. overall survival - OS) [12]–[14]. Szczególną grupą pacjentek stanowią kobiety, które nie zrealizowały jeszcze swoich planów prokreacyjnych i w wielu przypadkach mogą one skorzystać z zabezpieczenia płodności np. pobrania oocytów oraz operacji oszczędzających (ang. oncofertility) [15].

3.5. Patogeneza nowotworu

Początki opisów patogenezy raka jajnika sięgają lat 70 XIX wieku [16]. Klasyczna teoria powstawania nowotworu zaproponowana przez M. Fathalla wskazująca na rolę powtarzalnych uszkodzeń w trakcie owulacji miała wiele wad i próby doświadczalnego odtworzenia tego zjawiska nie powiodły się [17], [18]. Niemniej jednak wiele badań i wieloletnich obserwacji pośrednio potwierdza tę hipotezę. Wykazano ochronne działanie doustnej antykoncepcji, laktacji, ciąży czy wieku późnej menarche i wczesnej menopauzy. Z kolei wśród czynników ryzyka rozwoju OC opisuje się hormonalną terapię zastępczą (HRT)[19], [20]. Dubeau na podstawie obserwacji, że m.in. raki surowicze jajnika, jajowodu i otrzewnej są histologicznie identyczne zaproponował hipotezę embriologicznego pochodzenia guzów z przewodów Mullera lub na drodze transformacji mullerowskiej [21], [22]. Wypadkową tych doniesień jest teoria o źródle nowotworu w strzępkach jajowodu [23] – [25]. Obserwacja ta znalazła swoje zastosowanie w działaniach o charakterze prewencyjnym zapobiegających indukcji raka jajnika, polegającej na resekcji jajowodów w trakcie innych zabiegów ginekologicznych [26]. Pomimo rosnącej ilości dowodów na jajowodowe pochodzenie HGSOC badacze nie są zgodni co do pierwotnego źródła tego rodzaju nowotworu. Wydaje się, iż w chwili obecnej nie ma jednoznacznie zdefiniowanej dominującej teorii patogenezy raka jajnika. Tym nie mniej istotne są poszukiwania innych, potencjalnie prawdopodobnych szlaków indukujących proces nowotworzenia.

Na podstawie przeglądu piśmiennictwa w pracy przeglądowej analizowano rolę hormonów produkowanych w tkance tłuszczowej nazywanych adipokinami. Skupiono się na adiponektynie oraz leptynie. Wiele badań potwierdza znaczącą korelację pomiędzy obniżoną ekspresją adiponektyny, a zwiększoną leptyny w komórkach raka jajnika. Istnieją jednoznaczne dowody, że otyłość, a w konsekwencji przewlekły proces zapalny i towarzysząca mu synteza reaktywnych form tlenu biorą udział w patogenezie nowotworów [27] – [30]. Pomimo wielu

doniesień wskazujących na rolę otyłości w rozwoju OC, nie udowodniono jednoznacznego związku nadmiernej masy ciała z powstawaniem raka jajnika [20], [31], [32]. Z uwagi na powyższe w naszych badaniach również dokonano podziału pacjentek pod względem masy ciała, aby zweryfikować dotychczasowe obserwacje.

Przegląd literatury dotyczącej roli nadmiernej masy ciała w patogenezie raka jajnika został opracowany w publikacji: Baczewska, M.; Bojczuk, K.; Kołakowski, A.; Dobroch, J.; Guzik, P.; Knapp, P. Obesity and Energy Substrate Transporters in Ovarian Cancer—Review. Molecules 2021, 26, 1659.

Rozwój tkanki raka jajnika wymaga dostarczania energii, co jest możliwe dzięki interakcjom pomiędzy tkanką nowotworową a tkankami otaczającymi. Badania wykazały, że metabolizm większości komórek nowotworowych jest wysoce zależny od glukozy [33]. Obserwuje się zarówno procesy tlenowe jak i beztlenowe co czyni tkankę guza odporną na niekorzystne warunki w środowisku. Glikoliza nie tylko dostarcza ATP, ale również metabolitów zaangażowanych w biosyntezę aminokwasów i tłuszczy. Niektóre badania wykazały związek ekspresji białkowego transportera glukozy typu 1 (GLUT1) z gorszym rokowaniem u pacjentek z rakiem piersi i rakiem jelita grubego[34], [35]. Ponadto, wolne kwasy tłuszczowe odgrywają rolę budulcową dla komórek nowotworowych i mają swój udział w szlakach sygnałowych [36], [37]. Proliferacja komórek raka jajnika jest zależna od biodostępności wysokoenergetycznych lipidów/kwasów tłuszczowych dostarczanych w wyniku endogennej biosyntezy de novo i/lub importu z mikrośrodowiska. Badania na liniach komórkowych wykazały, że zachodzą istotne zmiany w wewnątrzkomórkowej homeostazie lipidowej w sytuacji niedoboru lipidów w środowisku zewnętrznym [38]. Kolejną grupą substancji niezbędnych do prawidłowego funkcjonowania komórki nowotworowej są aminokwasy. Transportery tych substancji - transportery aminokwasów (AATs) to białka błonowe, które pełnią różnorodne funkcje począwszy od neurotransmisji, przez utrzymywanie prawidłowej równowagi kwasowo-zasadowej. Biorą również udział w metabolizmie wewnątrzkomórkowym. Odpowiadają za transport aminokwasów z i do komórki, zatem wykorzystywane są zarówno w procesach anabolicznych jak i katabolicznych. Zaburzenia w funkcji AAT mogą skutkować kancerogenezą, rozwojem otyłości i cukrzycy [39]. Opisywano zwiększoną ekspresję neutralnego transportera dokomórkowego aminokwasów (LAT-1) w wielu nowotworach złośliwych. W metaanalizie poświęconej temu przekaźnikowi potwierdzono związek zwiększeniem poziomu LAT-1 u chorych na nowotwory a gorszym rokowaniem [40]. Nadal

brakuje jednak danych dotyczących znaczenia transporterów aminokwasów w nowotworach jajnika.

Innym zagadnieniem jest zjawisko tworzenia przerzutów. Rozsiew komórek nowotworowych zależy od zdolności przeżycia komórek nabłonkowych po ich oddzieleniu od zasadniczej tkanki guza. Komórki stają się oporne na apoptozę wskutek zwiększenia stężenia enzymów biorących udział w beta-oksydacji tj palmitoilotransferazy karnitynowej 1 (CPT1) [41]. Zarówno transportery glukozy, kwasów tłuszczowych jak i aminokwasów mają znaczenie w rozwoju, wzroście i przerzutowaniu komórek nowotworów złośliwych zatem mogą potencjalnie stanowić punkty uchwytu dla nowoczesnych terapii celowanych. Badania in vitro wykazały, że użycie inhibitorów dla wybranych transporterów wywoływało zależne od dawki/czasu hamowanie wychwytu kwasów tłuszczowych i wiązało się z obniżeniem proliferacji, zatrzymaniem cyklu komórkowego oraz apoptozą [42].

Metabolizm raka jajnika nie został do końca poznany. W literaturze można znaleźć opisy wielu przekaźników substratów energetycznych i ich znaczenia w nowotworach złośliwych. Dotychczas pomimo wielu badań nie udało się wyodrębnić ani dominującego substratu ani korespondującego transportera w raku surowiczym jajnika o wysokim stopniu złośliwości. Brakowało również kompleksowej pracy oryginalnej, w której porównano by ekspresję różnych transporterów energetycznych, oceniono korelacje i znaczenie kliniczne. Mając na uwadze wiele dowodów na różnice w metabolizmie tkanki nowotworowej i znaczenie transporterów substratów energetycznych zadecydowano o wykonaniu analiz, które zostały opisane w niniejszej rozprawie doktorskiej.

3.6 Rodzaje analizowanych transporterów substratów energetycznych z podziałem na substraty

3.6.1Transportery glukozy

Transportery glukozy są grupą glikoprotein błonowych występujących w komórkach ssaków. Do tej pory opisano 14 rodzajów transporterów glukozy kodowanych przez ludzki genom. Różnią się one lokalizacją w organizmie ssaków oraz sposobem transmisji glukozy. W kontekście nowotworzenia najlepiej poznane przekaźniki, obecne w wielu guzach nowotworowych to GLUT1, GLUT-3 i GLUT-4 [43] – [47]. Zarówno GLUT-1 jak i GLUT-3 transportują glukozę w sposób ciągły i niezależny od insuliny. Z kolei GLUT-4 jest

transporterem insulinozależnym. Nadal brakuje jednoznacznych dowodów na obecność zwiększonej ekspresji tych białek w tkankach raka surowiczego jajnika.

3.6.2 Transportery mleczanów

Transportery monokarboksylowe są to białka błonowe transportujące substancje zawierające w swojej budowie grupę karboksylową (np. kwas mlekowy, pirogronian, ketony). Występowanie w przyrodzie zjawiska Warburga, odkrytego przez laureata Nagrody Nobla Otto Warburga w 1920 r, które polega na przemianie glukozy do kwasu mlekowego w warunkach beztlenowych, jest źródłem wielu analiz doświadczalnych z użyciem transporterów transbłonowych mleczanów. Oprócz znanego potencjału tego substratu do produkcji energii przez komórki nowotworowe w procesie beta oksydacji wykazano, że MCT1 i MCT4 odgrywają kluczową rolę w utrzymaniu wewnątrzkomórkowego pH poprzez transport kwasów monokarboksylowych (takich jak mleczan, pirogronian i maślan) [48] – [50].

3.6.3 Transportery kwasów tłuszczowych i inne białka biorące udział w metabolizmie lipidów.

FABPs (białka wiążące kwasy tłuszczowe) jest to grupa transporterów substancji lipofilnych w komórkach. Obserwuje się zjawisko zwiększonej biodostępności wolnych kwasów tłuszczowych w środowisku wzmożonego metabolizmu jakim jest tkanka nowotworowa. Istnieje wiele doniesień na temat roli oksydacji kwasów tłuszczowych w progresji nowotworów złośliwych. Analizowano m.in. ekspresję FABP4 (białka wiążącego kwasy tłuszczowe 4) [51], [52], CD36 – (translokazy kwasów tłuszczowych kodowana przez gen CD36) [53], [54], FABP6 (białka wiążącego kwasy tłuszczowe 6) [51], [55], FABPpm (błonowego białka wiążącego kwasy tłuszczowe [56].

LPL (lipaza lipoproteinowa) jest to enzym niezbędny do hydrolitycznego uwalniania kwasów tłuszczowych z triacylogliceroli, które zawarte są w krążących lipoproteinach. Jest to zatem enzym pośrednio biorący udział w dostarczaniu substratów energetycznych, więc jego aktywność determinuje stopień zużycia kwasów tłuszczowych w komórkach nowotworowych. Z kolei syntaza kwasów tłuszczowych (FASN) jest enzymem, który katalizuje reakcję syntezy kwasów tłuszczowych, jest biomarkerem nadmiaru substancji odżywczych mającym znaczenie w powstawaniu insulinooporność. Najnowsze badania pokazują pośrednią rolę tego enzymu w progresji raka jelita grubego [57] [58].

3.6.4 Geny mitochondrialne potencjalnie istotne metabolicznie w raku jajnika

Mitochondria są to organella komórkowe, które obecne są w każdej komórce i odpowiadają za zasadniczą część produkcji ATP. Pomimo opisywanego wcześniej zjawiska Warburga, które opiera swoje założenia na przewadze procesów beztlenowych w komórce nowotworowej (co teoretycznie może być spowodowane upośledzeniem funkcji mitochondrialnych), większość badań sugeruje istotną rolę mitochondriów. Są one nie tylko producentem energii w postaci ATP, ale również pełnią funkcje sygnałowe i budulcowe. Ingerencja w geny mitochondrialne np. w mitochondrialny czynnik transkrypcyjny A (TFAM) skutkowała hamowaniem rozwoju nowotworu w mysim modelu raka płuca. Reaktywne formy tlenu (ang. reactive oxygen species ROS) produkowane w trakcie metabolizmu mitochondrialnego również okazują się niezbędne i są komponentą wielu nowotworowych szlaków sygnałowych[59]. W procesie syntezy energii bierze udział szereg enzymów, w tym β-HAD (ang. 3-hydroxyacyl-CoA dehydrogenase) katalizujący mitochondrialną beta oksydację lipidów. Jednym z głównych czynników regulujących proces biogenezy mitochondrialnej jest koaktywator 1-alfa receptora gamma aktywowanego przez proliferatory peroksysomów (PGC-1α). PGC-1α kontroluje między innymi ekspresję TFAM zaangażowanego w transkrypcję i replikację mitochondrialnego DNA. Rolę TFAM opisano również w patogenezie raka okrężnicy [60]. Oksydaza cytochromu C, podjednostka 4/1 (COX4/1) jest końcowym enzymem łańcucha oddechowego. Istnieje szereg badań, które potwierdziły związek zmian ekspresji tego enzymu w patogenezie raka tarczycy, oporności na chemioterapię w glejaku [61] czy zaburzeniach apoptozy np. w raku szyjki macicy [62].

3.6.5 Transportery aminokwasów

Aminokwasy są niezbędne do przeżycia i proliferacji komórek nowotworowych, ponieważ pełnią funkcję nie tylko składników budulcowych komórek, ale również substratów w syntezie innych związków metabolizowanych następnie w cyklu kwasów trójkarboksylowych (TCA). W związku z powyższym, potencjalny wpływ kontroli dostępności i metabolizmu aminokwasów w mikrośrodowisku guza staje się coraz ważniejszy. W ostatnich latach opracowano terapie celowane, które mają na celu zakłócenie dostępu aminokwasów. Przykładem może być hamowanie proliferacji komórek raka żołądka in vitro przy udziale microRNA-126 jako regulatora transkrypcji genu *LAT-1* [63].

W piśmiennictwie można znaleźć opracowania dla genów: *LAT1* (Na+-niezależnego

transportera dokomórkowego aminokwasów: leucyny, izoleucyny, fenyloalaniny, metioniny, histydyny, tryptofanu, waliny i tyrozyny [64]), *ASCT2* (Na+- zależnego transportera nazwanego akronimem od aminokwasów: alaniny, seryny, cysteiny) oraz *SNAT1* (neutralnego transportera aminokwasów sprzężonego z sodem typu 1 [65]).

Szczegółowy przegląd literatury poświęcony transporterom substratów energetycznych został zawarty w publikacji Baczewska, M.; Bojczuk, K.; Kołakowski, A.; Dobroch, J.; Guzik, P.; Knapp, P. Obesity and Energy Substrate Transporters in Ovarian Cancer—Review. Molecules 2021, 26, 1659

4. Cel pracy

Istnieje wiele dowodów na zasadność poszukiwania sposobów leczenia celowanego u pacjentów, którzy posiadają agresywny wariant raka surowiczego jajnika. W dalszym ciągu nie wyodrębniono preferowanego substratu energetycznego w komórkach raka jajnika, co mogłoby znacząco poprawić rokowanie pacjentek. Znalezienie różnic w metabolizmie komórek nowotworu złośliwego jajnika, a komórkami zdrowymi pozwoliłoby lepiej zrozumieć biologię guzów i stanowić podłoże do dalszych badań oraz poszukiwań terapii celowanych, które mogłyby stanowić uzupełnienie dla klasycznych form leczenia.

Szczegółowe cele przeprowadzonych badań obejmowały:

1. Ocenę ekspresji genów kodujących transportery wybranych substratów energetycznych w tkance nowotworowej jajnika.

2. Analizę zależności pomiędzy ekspresją genów a wybranymi parametrami klinicznymi i biochemicznymi tj. obecność przerzutów, wielkość guza pierwotnego, czas hospitalizacji, BMI (podział na nadwagę i otyłość), stężenie CA 125 (antygen nowotworowy 125), ilość płytek krwi na mm^3, stężenie fibrynogenu, potasu, hormonu tyreotropowego (TSH).

3. Analizę czasu przeżycia w odniesieniu do ekspresji badanych genów na podstawie danych zawartych w ogólnodostępnej bazie TCGA (The Cancer Genome Atlas).

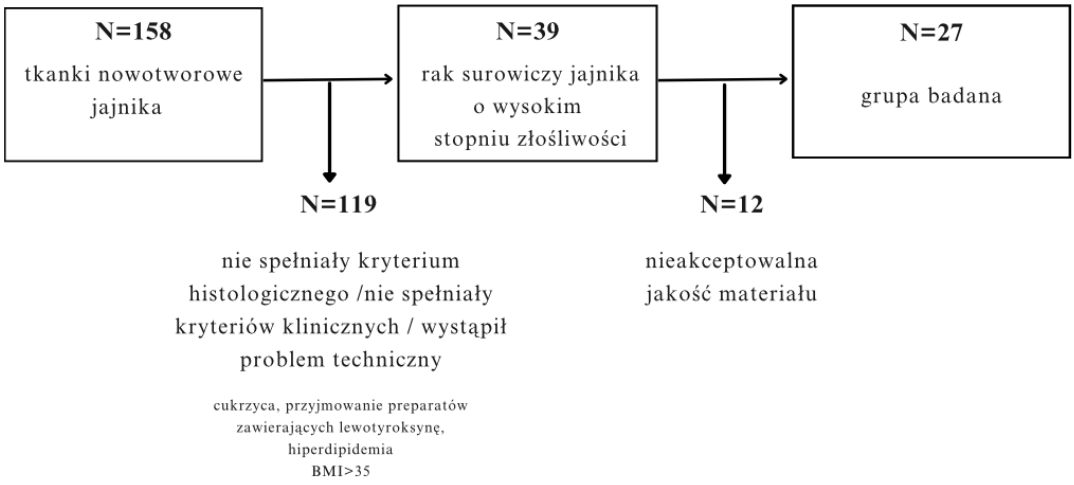
5. Materiały i metody

Szczegółowe informacje dotyczące metodyki badań można znaleźć w pracy: Baczewska, M.; Supruniuk, E.; Bojczuk, K.; Guzik, P.; Milewska,P.; Kononczuk,K.; Dobroch, J.; Chabowski, A.; Knapp, P. Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications. Int. *J. Mol. Sci*. 2022, 23, 8968.

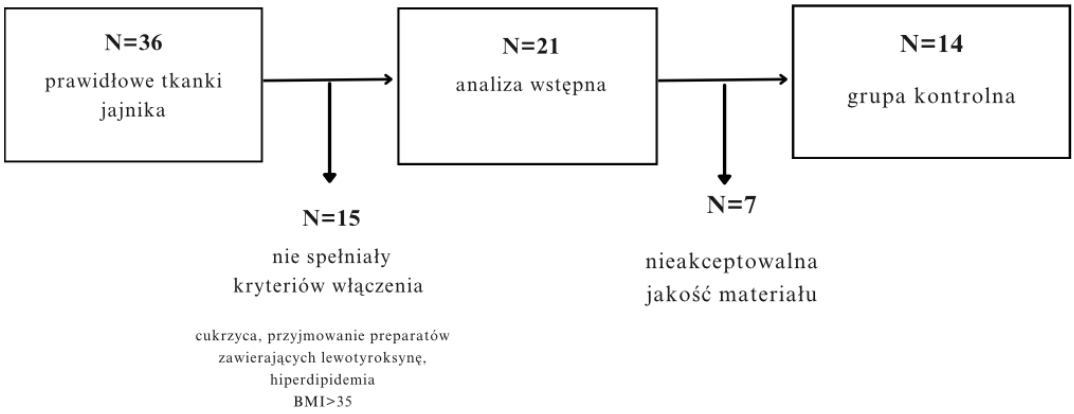
5.1 Charakterystyka grupy badanej i kontrolnej

Próbki zostały zabezpieczone przez zespół pracowników Biobanku Uniwersytetu Medycznego w Białymstoku zgodnie ze ściśle określonymi procedurami. W pierwszym etapie przeanalizowano profil 158 pacjentek leczonych z powodu raka jajnika w Klinice Ginekologii i Ginekologii Onkologicznej i Uniwersyteckim Centrum Onkologii w latach 2017-2021. Wyselekcjonowano podtyp histologiczny raka surowiczego o wysokim stopniu złośliwości i zastosowano kryteria wyłączenia (otyłość BMI >35 kg/m^2, cukrzyca, terapia lewotyroksyną i hiperlidemia; Rycina 1). Grupa badana składała się z 27 pacjentek. 23 z nich prezentowało III lub IV stopień zaawansowania raka jajnika wg FIGO. Cztery pacjentki były nosicielkami mutacji BRCA1/2. Pacjentki podzielono dodatkowo pod względem manifestacji klinicznej. Część pacjentek prezentowała mały guz pierwotny, gdzie jednoczasowo obserwowano rozsiew drobnoguzkowy w jamie brzusznej szczególnie do sieci większej z towarzyszącym wodobrzuszem. Druga część pacjentek charakteryzowała się przerzutami do okolicznych węzłów chłonnych i brakiem obecności drobnoguzkowego rozsiewu.

Grupę kontrolną stanowiły tkanki zdrowego jajnika od pacjentek, które były operowane w Klinice Ginekologii i Ginekologii Onkologicznej z powodów innych niż nowotworowe (Rycina 2).



Rycina 1. Dobór grupy badanej.



Rycina 2. Dobór grupy kontrolnej.

5.2 Analiza ekspresji genów na poziomie mRNA z wykorzystaniem ilościowej reakcji łańcuchowej polimerazy w czasie rzeczywistym (real-time quantative PCR)

Całkowite RNA zostało wyizolowane z tkanek jajnika przy pomocy NucleoSpin RNA Plus Kit z RNase-free DNase I (Ambion, Thermo Fisher Scientific, Waltham, MA, USA) zgodnie z protokołem producenta. Przeprowadzono spektrofotometryczną ocenę ilościową i jakościową RNA (OD ratio of 260/280 and 260/230). Całkowite RNA (1 μg) posłużyło za matrycę do syntezy komplementarnej nici cDNA (EvoScript universal cDNA master kit (Roche Molecular Systems, Boston, MA, USA). Ilościowa reakcja łańcuchowa polimerazy w czasie rzeczywistym (qRT-PCR, *real-time quantative PCR*) została wykonana przy użyciu LightCycler 96 System z wykorzystaniem zestawu FastStart essential DNA green master (Roche Molecular Systems). Zastosowano następujące parametry: 15 s denaturacja w 94 ◦C, 30 s amplifikacja w 60 ◦C w przypadku *CD36/SR-B2, FATP1, FATP4, FABPpm*, *FABP4, GLUT1, GLUT4, FASN* i *β-actin* lub 61 ◦C w przypadku *MCT1, MCT4, LAT1, ASCT2, SNAT1, PGC-1α, TFAM*, *β-HAD, COX4/1* oraz *LPL*, a następnie 30 s elongacja w 72 ◦C. Specyficzność uzyskanych produktów zweryfikowano w oparciu o krzywą topnienia przeprowadzoną na zakończenie każdego cyklu reakcji. W celu normalizacji otrzymanych wyników równolegle oceniono ekspresję genu referencyjnego, β-aktyny. Wyniki zostały obliczone na podstawie matematycznego modelu Pfaffl [66]

5.3 Analiza statystyczna danych zawartych w bazach TCGA i GTEx

Uzyskane wyniki badań laboratoryjnych porównano z danymi zdeponowanymi w ogólnodostępnej bazie TCGA (The Cancer Genome Atlas). TCGA należy do prowadzonych na szeroką skalę międzynarodowych, wieloośrodkowych projektów badawczych, których celem jest charakterystyka molekularna ponad 20.000 podstawowych nowotworów. Analizie statystycznej poddano dane dotyczące ekspresji genów w próbkach uzyskanych od 426 pacjentów z rakiem jajnika przy użyciu pakietu GEPIA (ang. Gene Expression Profiling Interactive Analysis) w odniesieniu do prób kontrolnych zawartych w bazie GTEx (ang. Genotype-Tissue Expression project). Liczebność grupy kontrolnej wyniosła 88 pacjentek [67].

5.4 Analiza statystyczna

Do analizy statystycznej użyto oprogramowania GraphPad 8.0. Stosowano testy Shapiro-Wilka (w celu oceny rozkładu normalnego) oraz Levene'a (test jednorodności wariancji). W celu

porównania 2 grup użyto testu t-studenta lub U Manna-Whitneya. Do porównywania większej ilości grup zastosowano jednoczynnikową analizę wariancji (ANOVA) lub test Kruskala-Wallisa oraz odpowiedni test post-hoc. Korelacje pomiędzy badanymi parametrami oceniano za pomocą współczynnika korelacji rang Spearmana. Celem oceny przeżywalności zastosowano krzywe Kaplana-Meiera oraz test log-rank. Różnice uznano za istotne statystycznie przy $p < 0.05$.

6. Wyniki

Szczegółowe informacje dotyczące wyników badań zostały zawarte w pracy: Baczewska, M.; Supruniuk, E.; Bojczuk, K.; Guzik, P.; Milewska, P.; Kononczuk, K.; Dobroch, J.; Chabowski, A.; Knapp, P. Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications. Int. J. Mol. Sci. 2022

6.1 Ekspresja genów transporterów substratów energetycznych

W odniesieniu do transporterów kwasów tłuszczowych, Zaobserwowano zmniejszenie ekspresji *CD36/SR-B2* oraz *FATP1* w raku jajnika w porównaniu do kontroli (−37%, −62%). Poziom mRNA *FABPpm* był podwyższony (+89%), podczas gdy ekspresja *FATP4* pozostała względnie stabilna w obydwu grupach.

Zawartość transkryptu cytozolowego białka *FABP4* była obniżona (−93%) w tkance nowotworowej jajnika. Nie odnotowano różnic pomiędzy ekspresją *FASN*, co wskazuje na porównywalny poziom syntezy de novo kwasów tłuszczowych w tkance nowotworowej i w kontrolnej. Ekspresja *LPL*, zaangażowanej w proces uwalniania kwasów tłuszczowych z triacylogliceroli zawartych w krążących lipoproteinach, była obniżona o 78% w komórkach raka.

W próbkach HGSOC zaobserwowano zwiększenie ekspresji *GLUT1,* a zmniejszenie *GLUT4*. Wyniki te były zgodne z dotychczasowymi badaniami, które wskazywały na przewagę GLUT1 w tkance nowotworowej nad innymi transporterami [68], [69]. Zwiększenie transkryptu odnotowano również dla *MCT4.* W przypadku *MCT1* nie wykazano zmian. Należy zaznaczyć, że relatywna zawartość mRNA *GLUT1* i *MCT4* w grupie kontrolnej była najniższa ze wszystkich badanych transporterów, natomiast zwiększyła się w największym stopniu w grupie badanej w porównaniu do pozostałych genów.

Ponadto, zawartość mRNA *LAT1* była porównywalna w obydwu grupach, natomiast wykazano obniżenie ekspresji *ASCT2*. Co ciekawe, ekspresja transportera *SNAT1* była znacznie zwiększona w tkance nowotworowej.

6.2 Ekspresja genów mitochondrialnych

Zaobserwowano istotne statystycznie obniżenie ekspresji transkryptu *β-HAD* w raku jajnika (−60%) w porównaniu do grupy kontrolnej. Nie wykazano różnic w stężeniach mRNA *PGC-1α*, *TFAM* oraz *COX4/1* pomiędzy tkanką nowotworową jajnika i kontrolną.

6.3 Zależność pomiędzy a ekspresją wybranych genów a wybranymi parametrami klinicznymi pacjentów

Ekspresja analizowanych genów nie korelowała z klinicznym stopniem zaawansowania nowotworu wg klasyfikacji FIGO, inwazją węzłów chłonnych czy sieci większej. Niemniej jednak zaobserwowano znacznie zwiększoną ekspresję *PGC-1α* mRNA (+1400%) oraz obniżoną ekspresję *MCT4* (-57%) w relatywnie małych guzach, które cechowały się skłonnością do tworzenia przerzutów do sieci większej, w porównaniu do tkanek nowotworowych, które miały tendencję do inwazji węzłów chłonnych.

W grupie z nadwagą (BMI wyższe niż 25 kg/m^2) nie obserwowano różnic w ilości transkryptu badanych genów. W grupie >30 kg/m^2 odnotowano zwiększoną ekspresję *FABPpm*, *PGC-1α*, *FASN* w porównaniu do pacjentów o prawidłowej masie ciała.

6.4 Korelacje

Zaobserwowano zarówno pozytywne jak i negatywne korelacje w ekspresji badanych transporterów. W komórkach raka jajnika zaobserwowano istotne korelacje pomiędzy *FABP4* i *FABPpm* ($p = 0.009$, $r = 0.490$), *FATP4* i *MCT1* ($p = 0.009$, $r = 0.489$), *FATP4* i *FABPpm* ($p = 0.0004$, $r = 0.636$), oraz *SNAT1* i *GLUT1* ($p = 0.005$, $r = 0.527$). Ponadto stwierdzono pozytywne korelacje pomiędzy ekspresją *FABPpm* i BMI, *GLUT*1 i stężeniem glukozy w osoczu oraz *LAT1* i objętością guza.

Szczegółową analizę wraz z graficzną prezentacją w postaci statystycznej mapy ciepła „heat map" można znaleźć w pracy: Baczewska, M.; Supruniuk, E.; Bojczuk, K.; Guzik, P.; Milewska, P.; Kononczuk, K.; Dobroch, J.; Chabowski, A.; Knapp, P. Energy Substrate

Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications. Int. J. Mol. Sci. 2022.

6.5 Ocena wartości prognostycznej wybranych genów na podstawie danych zawartych w bazie TCGA

Celem określenia związku pomiędzy ekspresją genów związanych z metabolizmem raka jajnika a danymi klinicznymi, przeanalizowano przeżycie całkowite (ang. overall survival, OS), czas przeżycia wolny od progresji choroby (ang. progression-free survival, PFS) i stopień zaawansowania klinicznego dla każdego genu. W analizie przeżycia całkowitego wyższe wartości *FABP4* i *LPL* oraz niższe *TFAM* wiązały się z gorszym rokowaniem. Wzrost ekspresji *FABP4, PGC-1α* oraz *COX4/1* korelował z krótszym PFS, natomiast wzrost *GLUT4* i *TFAM* z dłuższym. Analiza w oparciu o TCGA pozwoliła również określić zmiany ekspresji związane ze stopniem zaawansowania klinicznego nowotworu. Spośród ocenianych genów, poziom ekspresji *FABPpm, FABP4, FASN, GLUT1* i *TFAM* miał związek z klinicznym stadium raka jajnika.

6.6 Podsumowanie

Wyniki badań laboratoryjnych oraz analiza danych z bazy TCGA potwierdzają znaczne różnice pomiędzy metabolizmem komórek prawidłowych oraz nowotworowych, zatem należy nadal prowadzić badania na większych grupach, wieloośrodkowo, aby znaleźć zastosowanie tych zjawisk w praktyce klinicznej, i podejmować próby zastosowania substancji hamujących jako terapii celowanych w leczeniu nabłonkowego raka jajnika.

7. Wnioski

- Wzrost ekspresji *FABPpm*, *SNAT1* oraz *GLUT1* w HGSOC potwierdza zwiększone zużycie podstawowych substratów energetycznych w komórkach guza.
- Największy wzrost ekspresji odnotowano w przypadku *GLUT1* w tkance nowotworowej jajnika, co wskazuje na przewagę wykorzystania glukozy jako podstawowego substratu energetycznego w HGSOC.
- Komórki raka jajnika, dzięki zwiększonej ekspresji *MCT4*, mogą utrzymywać sprzyjające im kwasowe środowisko, co może wpływać na złośliwość nowotworu.
- Wzrost ekspresji *SNAT1* sugeruje istotny udział glutaminy w progresji HGSOC.
- Ekspresja poszczególnych genów zależała zarówno od klinicznej manifestacji nowotworu, obecności przerzutów, jak i BMI pacjentek.
- Pacjentki z wysokim poziomem ekspresji *FABP4*, *PGC-1α* oraz *COX4/1* cechował krótszy czas przeżycia wolny od progresji, natomiast wzrost *GLUT4* i *TFAM* wiązał się z dłuższym czasem przeżycia wolnym od progresji nowotworu.

8. Publikacja nr 1.

Baczewska Marta, Bojczuk Klaudia, Kołakowski Adrian, Dobroch Jakub,

Guzik Paweł, Knapp Paweł

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Obesity and Energy Substrate Transporters in Ovarian Cancer-Review.

Molecules

2021 Mar 16;26(6):1659.

IF= 4,927 MEiN=140 pkt.

Białystok, 2023

molecules

MDPI

Review

Obesity and Energy Substrate Transporters in Ovarian Cancer—Review

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Abstract: Ovarian cancer is the seventh most common cancer in women. It is characterized by a high mortality rate because of its aggressiveness and advanced stage at the time of diagnosis. It is a nonhomogenous group of neoplasms and, of which the molecular basics are still being investigated. Nowadays, the golden standard in the treatment is debulking cytoreductive surgery combined with platinum-based chemotherapy. We have presented the interactions and the resulting perspectives between fatty acid transporters, glucose transporters and ovarian cancer cells. Studies have shown the association between a lipid-rich environment and cancer progression, which suggests the use of correspondent transporter inhibitors as promising chemotherapeutic agents. This review summarizes preclinical and clinical studies highlighting the role of fatty acid transport proteins and glucose transporters in development, growth, metastasizing and its potential use in targeted therapies of ovarian cancer.

Keywords: ovarian cancer; obesity; cancer progression; targeted therapy; lipids

Citation: Baczewska, M.; Bojczuk, K.; Kołakowski, A.; Dobroch, J.; Guzik, P.; Knapp, P. Obesity and Energy Substrate Transporters in Ovarian Cancer—Review. *Molecules* **2021**, *26*, 1659. https://doi.org/10.3390/molecules26061659

Academic Editor: Farid Chemat

Received: 2 February 2021
Accepted: 12 March 2021
Published: 16 March 2021

1. Introduction

Ovarian cancer (OC) is the seventh most common cancer in women and the most common cause of death from gynecological cancers, with a 5-year survival rate below 45% [1,2]. 90% of ovarian cancers are epithelial cancers, the most common, of which are high-grade serous ovarian cancer (HGSOC), known among healthcare professionals as the silent killer [3]. According to US data from 2016 covering the entire female population in the US for every 100,000 women, 11 new ovarian cancer cases were reported, and 7 died of cancer. Late diagnosis of advanced disease is the main cause of poor prognosis. In the early stages of the disease, patients do not present any symptoms; the screening test does not exist. Over the last two decades, ovarian cancer rates have decreased in North America and Europe [4]. Approximately half of the epithelial ovarian cancers (EOC) had defects in DNA repairing systems, while 96% of HGSOC tumors have TP53 mutation and present impaired apoptosis [5,6]. The recent studies focus on the investigation of the metabolic basis of OC. Observation of diverse clinical conditions in patients with an equal histopathological status suggests the potential difference between preferred energy substrates. Factors as hypoxia, oxidative stress or inflammation generally redirect the cell metabolism into anaerobic processes and enhance the role of glucose. However, the bioavailability of free fatty acids increases analogically in a neoplastic environment. The aim of this work is to present the current scientific approach to OC metabolism and the potential clinical application.

2. From Diagnosis to Setting a Proper Treatment Plan in OC

2.1. Signs and Symptoms

Although ovarian cancer can occur in younger women, it has a predisposition to develop in women over 50 and with menopause, which means that as life expectancy increases, the number of cases diagnosed increases each year. The vast majority of ovarian cancers are diagnosed at an advanced stage due to the asymptomatic course of the early-stage and nonspecific symptoms of the advanced stage [3,7]. The presence of symptoms, such as pelvic pain, constipation, diarrhea, nausea, urinary problems, early satiety, suggest performing a physical examination and transvaginal ultrasounds initially [8,9].

2.2. Diagnosis

Thereafter, ovarian cancer diagnosis includes contrast computed tomography (CT) or magnetic resonance imaging (MRI) of the chest, abdomen, and pelvis to determine cancer stage and the presence of metastases. It is also advisable to test tumor markers, such as CA-125, CEA and CA 19–9, in order to exclude other causes of abdominal mass symptoms different from ovarian cancer. Additionally, the severity of the disease and potential resectability should be elucidated prior to laparotomy. Laparoscopic evaluation of the abdominal cavity or percutaneous biopsy in the case of disseminated disease should be considered. Examination of abdominal fluid samples in patients with ascites can also be used for cachectic patients but is less reliable [10]. It is vital to provide genetic counseling for women with a family history associated with a risk of harmful BRCA1/2 mutations and HR deficiency (HRD), which increases the likelihood of developing ovarian cancer [11].

2.3. Surgical Treatment and Chemotherapy

Nowadays, the golden standard in the treatment is debulking cytoreductive surgery combined with platinum-based chemotherapy (cisplatin, carboplatin or oxaliplatin) and a taxane in 4–8 cycles. Over the last two decades, guidelines have changed significantly [12]. In advanced stages of OC, neoadjuvant therapy may be required before the surgery in order to reduce tumor mass [8]. Cancer recurrence and platinum resistance are common burdens in women diagnosed in the advanced stage. The scientific findings in the area of molecular and genetic alternations in OC suggest that the potential beneficial effect of targeted therapies should be inevitably evaluated [13].

Vasculogenesis and angiogenesis mediated by vascular epithelial growth factor (VEGF) has a prominent meaning in the epithelial ovarian cancer development and spread. The tumor blood vessels are more prone to VEGF effects than normal ones. Inhibition of vascular epithelial growth factor receptors (VEGFR), highly expressed in OC, decreases tumor vessels or metastases formation and cancer progression. Monoclonal antibody bevacizumab inhibits tumor VEGFR [11]. AURELIA trial reveals that adding bevacizumab to conventional chemotherapy in platinum-resistant OC enhances response to the treatment and increases progression-free survival [14].

2.4. Maintenance Treatment

Novel and constantly evolving treatments for primary and recurrent ovarian cancer include angiogenesis inhibitors, poly (ADP-ribose) polymerase (PARP) inhibitors and immunotherapy agents [6,15]. Furthermore, PARP inhibitors, such as olaparib, niraparib and rucaparib, play an essential modulatory role by inhibiting DNA repairing systems [1]. Since BRCA mutations lead to cells' DNA double-strand breaks, PARP inhibitors can cause the death of those cells by leaving their DNA damaged [13]. A current state of knowledge shows that PARP inhibitors have an application in the treatment of patients with BRCA1/2 mutations and recurrent ovarian cancer [1]. There is a strong need to elucidate novel therapies based on inhibiting cell proliferation and angiogenesis because of their potential application.

3. Obesity and Ovarian Cancer

Obesity poses a threat for diverse tumor development and is correlated with dismal prognosis [16]. The high concentration of adipocytes in the human organism results in adipose tissue impairment, which leads to immune and hormonal alternations in the microenvironment that is a fundamental part of carcinogenesis [16]. However, the excessive visceral fat distribution is suspected to increase the likelihood of cancer development, but a general higher fat concentration in the whole human body is not correlated with this risk [17]. Besides the widespread conviction that the OC is an obesity-related neoplasm, the meta-analyses do not confirm this theory. Nevertheless, the association between increased body weight and ovarian cancer exists in the premenopausal period. High-grade invasive serous tumors, the most fatal subtype, in this study were not associated with BMI [18]. Bae et al. also showed that BMI at diagnosis could not be a prognostic factor for the survival of ovarian cancer patients [19]. However, there are numerous studies describing an increased risk of ovarian cancer in obese women. Foong et al. demonstrated obesity as a potentially modifiable malignant factor in ovarian cancer in their meta-analysis [20]. Moreover, the literature reviews show conflicting reports on correlations between obesity and ovarian cancer, so more research is required to confirm this thesis. It is worth mentioning that the main methods of obesity measurement in the above-mentioned works were BMI and WHR. It is crucial that those indexes do not provide data on fat distribution and may vary depending on the clinical condition. In women suspicious of OC, the WHR is not adequate either due to ascites or mass in the abdomen. Dual-energy X-ray absorptiometry seems to be an adequate method to evaluate the exact amount of visceral fat but is hardly accessible. It is a high-quality alternative to the reference methods of measuring visceral adipose tissue, which is CT and MRI [21,22]. There are studies suggesting that future research should be more precise, unified and include features as the histological type and menopausal status [23].

The recent studies focus on the investigation of the metabolic basis of OC. The observation of diverse clinical conditions in patients with an equal histopathological status suggests the potential difference between preferred energy substrates. It is commonly known that cancer cells have increased requirements for ATP production and higher energy supply. Warburg et al. observed that malignant cell transformation can lead to higher glucose utilization via glycolysis even under normoxia [24]. Furthermore, other diverse coexistent factors involved in tumor development like hypoxia, oxidative stress or inflammation also redirect the cell metabolism into anaerobic processes and enhance the role of glucose [25,26]. Since cancerous cells derive energy mostly from glycolysis, they have a predilection to elevated glucose utilization. From the observations mentioned above, it can be proved that the cancer cells alter the expression of glucose transporters in order to increase glucose influx [27]. It can be observed that the overexpression of different facilitative glucose transporters (GLUT) has been discovered in diverse cancer tissues. Glucose transporter 1 (GLUT 1) is characteristic of liver, pancreas, kidney, lung and glucose transporter 3 (GLUT 3) of lung and ovarian neoplasms. Unfortunately, the higher presence of GLUTs in cancer tissues is not precisely evaluated, and further research should be conducted to provide more detailed data [28]. However, the bioavailability of free fatty acids (FFA) increases analogically in a neoplastic environment. Visceral adipose tissue surrounding tumors has a changed phenotype induced by cancerous cells. Cancer-associated adipocytes produce FFA and adipokines [26]. FFA can be a source of energy for cancerous tissues by fatty acid oxidation but also occupy a central part in a cell structure synthesis and mediate by fatty acid-binding protein (FABP) signaling pathways involved in cancer development [29]. Moreover, overexpression of fatty acid-binding protein 4 (FABP4) was observed in different cancers, e.g., prostate, bladder, renal cell carcinoma and also ovarian neoplasms. FABP4 is suspected to be a potential point of alternative treatments for cancers associated with the adipose microenvironment [30].

4. Glucose Metabolism in Cancer Cells

It is widely known that malignant transformation can lead to increased metabolism [27]. Furthermore, malignant cells are characteristic of higher glucose consumption via anaerobic processes, such as glycolysis even under normoxia, which is known as the Warburg effect [31]. Anaerobic glucose consumption results in less ATP production than oxidative phosphorylation [32]. Moreover, diverse environmental factors, such as hypoxia, inflammation, stress and lack of nutrients, also shift cancer metabolism into anaerobic. As a result of the mentioned observations, it is known that cancer cells require a huge amount of glucose [25,33,34]. Glucose influx into the cell is the first limiting process in the glycolysis pathway in non-malignant and cancerous tissues mediated by facilitative glucose transporters across the plasma membrane [27,35]. In the current state of knowledge, there are 14 GLUTs in mammalian cells involved in glucose transport, which have diverse functions in glucose uptake and also vary in their location in mammalian tissues, regulation and their affinities for glucose [36,37]. There are a few GLUTs overexpressed in cancer cells involved in glucose uptake, especially GLUT 1–4, in order to elevate glucose uptake [36,38]. Moreover, alternations in GLUTs expression in cancers are caused by diverse pathways. Influence of hypoxic environment on cancerous cells results in the inhibition of hypoxia-induced factor (HIF) alfa and beta degradation, which in this condition bind to hypoxia–response elements (HRE) and induce transcription of glycolytic genes like GLUT 1 and 3 [27,39,40]. PI3K and Akt pathway and p53 mutation also contribute to enhanced transcription of GLUT 1 and 3 [40]. The translocation of glucose transporter 4 (GLUT 4) to the plasma membrane in insulin-dependent tissues is related to insulin stimulation; however, factors, which induce the expression of GLUT 4 in cancerous tissues should be assessed [40]. Taking into account that malignant cells are highly dependent on glycolysis as the main way of energy production and that many factors regulate glucose uptake, there is the likelihood that the inhibition of proteins involved in glycolysis, such as glucose transporters, can have beneficial effects in cancer therapy [33].

4.1. A Broad Role of GLUT 1 in Cancer Development

GLUT 1 is abundantly found in the brain and erythrocytes, but also in adipocytes, liver and muscles and plays a vital role in basal glucose influx into the cell [27,41]. Furthermore, GLUT 1 is also regarded as a prerequisite in diverse processes of cancer development [42]. First of all, GLUT 1 is involved in enhanced glucose influx in malignant cells induced by genetic alternations, growth factors and also hypoxia. GLUT 1 also occupies a central part in energy production necessary for the proliferation of cancer cells [28,40]. Nogushi et al. proved that the presence of antisense GLUT 1 in mice resulted in the reduction of tumor growth with concomitant alternations in the cell cycle and lower glucose influx observed in a gastric carcinoma cell line with antisense GLUT 1. These observations confirm that the expression of GLUT 1 is a fundamental part of processes involved in tumor development [43]. Moreover, Tsukioka et al. revealed that there is a remarkable correlation between GLUT 1 expression and VEGF in EOC. GLUT 1 is possibly involved in new vessel formation in EOS via VEGF, which is a potent factor of neovascularization in EOC [42,44,45]. Additionally, GLUT 1 is also suspected to be a crucial factor in metastasis formation. Ito et al. detected that enhanced expression of GLUT 1 in rhabdomyosarcoma cell line was coupled with an elevated level of MMP-2 protein targeting degradation of collagen in the extracellular matrix and suspected to regulate cancer cell migration and invasion [46–48]. All of the observations above confirm that glucose uptake mediated by GLUT 1 plays a crucial role in cancer development, especially EOC, as well as in metastasis formation.

4.2. GLUT 1 in OC

The most prominent issue worth mentioning is that OC significantly varies from other types of cancers. OC is more prone to forming metastases by the intraperitoneal spread. Taking this into account, it can be supposed that OC cells and intraperitoneal metastases are more deprived of oxygen supply if they are located far away from blood vessels. Kalir et al.

showed that expression of GLUT 1 was observed in 96% of the human OC, lower expression of GLUT 1 was detected in a borderline tumor, and local expression of GLUT 1 was also observed in villus adenomas with the hazard of malignant transformation [49]. However, expression of GLUT 1 was observed only in 57% of specimens from the human esophageal squamous cell carcinomas in 60% of the human gastric cancer samples [24,50]. Higher expression of GLUT 1 in OC induced by hypoxia is thought to be an adaptive mechanism to oxygen deprivation observed in OC [49]. Furthermore, other research also indicates that that GLUT 1 is highly upregulated in malignant OC [51]. Cantuaria et al. revealed in their study the presence of GLUT 1 in 101 out of 103 specimens of the human EOC. Their findings also proved significant alternations in GLUT-1 expression in malignant rather than borderline and benign tumors [35]. In other studies, the expression of GLUT 1 was observed in 98.7% of the human EOC [44]. A significant increase in expression of GLUT 1 was also observed in well-differentiated OC [35]. There is also a vast majority of studies that show that there is a correlation between overexpression of GLUT 1 and the histological type of OC. GLUT1 is abundantly expressed in serous adenocarcinoma; however, expression of these transporters is decreased in clear cell adenocarcinoma [35,42,44,51,52]. There is also a correlation between overexpression of GLUT 1 and stage of OC. GLUT 1 is upregulated in an advanced stage of OC rather than in the early stages, but that observation was only detected in serous adenocarcinomas [33,44,51,53].

GLUT 1 is regarded as a prognostic factor and associated with dismal prognosis in some cancers, e.g., lung cancer, colon cancer, gastric cancer, renal [52,54–56]. Yin et al. revealed that the positive effect of the treatment was lower in gastric cancer with expression with GLUT 1 [55]. Some data indicate there is a possible connection between overexpression of GLUT 1 and prognosis in OC; however, that statement is unclear [51,53]. Cho et al. showed that overexpression of GLUT 1 in EOS is a prognostic factor of unfavorable prognosis, but assessing this marker has some analytic burdens [51]. Semaan et al. showed that advanced stages of OC with concomitant overexpression of GLUT 1 are less prone to chemotherapy, but one marker is not an adequate predictor in EOC [42]. Xintaropoulou et al. proved that there is no remarkable correlation between the expression of GLUT 1 in OC and patient survival [33]. Tsusioka showed that the evaluation of GLUT 1 expression in OC is inadequate for predicting prognosis because other factors like age, stage of cancer and histological type have a far more prognostic value for survival in OC [44].

Summing up, OC is highly dependent on glucose consumption, and overexpression of GLUT 1 is more obvious than in other cancers. Some types of OC, such as serous adenocarcinoma and advanced stages of OC, have a predilection for overexpressing GLUT 1. The application of novel glycolytic inhibitors as an additive therapy to conventional chemotherapy may have the clinical application [33,57,58]. However, the expression of GLUT 1 is not regarded as an adequate predictor of patient survival in OC. Understanding the fact that OC is highly reliant on glycolysis as a source of energy and that GLUT 1 plays a significant role in cancer development, some researchers checked whether the inhibition of GLUT 1 results in alternations in cancer cell proliferation and suppression of tumor growth. Some studies indicate that the inhibition of GLUT 1 has a potential clinical application in OC treatment if there is a significant overexpression of GLUT 1 in OC. Shin et al. proved that ciglitazone, an antidiabetic drug, can also play a crucial role in the inhibition of tumor proliferation in vitro by altering glucose uptake mediated by GLUT 1, but also by changing the amount of GLUT 1 in the plasma membrane. Furthermore, it was also observed that the application of ciglitazone in mice in vitro resulted in OC size reduction [57]. Ma et al. revealed that the application of a GLUT 1 inhibitor BAY-876 in OC in both in vitro and in vivo models in mice resulted in a 50–71% decrease in tumor growth and also reduction of glucose utilization rate [58]. Other research revealed that administration of STF 31, novel GLUT 1 inhibitor and metformin to conventional chemotherapy caused the amelioration of therapy effects in both platinum-sensitive and resistant OC [33]. All of the information above confirms that the inhibition of glucose uptake mediated by GLUT 1 plays a signifi-

cant role in the suppression of energy production in OC. The administration of GLUT 1 inhibitors can reduce cell proliferation and cancer growth. Resistance to platinum poses a threat in conventional OC chemotherapy, and the fact that platinum-resistant OC is still prone to glycolytic pathway inhibitors can cause beneficial development of conventional treatment in OC.

4.3. A Potential Role of GLUT 3 in OC and Other Cancers

The numerous research indicates an inevitable role of GLUT 1 in cancer development, whereas a precise function of other facilitative glucose transporters in that process is still not well understood [34]. GLUT 3 is also abundantly found in tissues with higher requirements for glucose, e.g., the brain, because of its higher affinity for glucose and plays a crucial role in basal glucose influx [41,59]. The presence of GLUT 3 was also detected in diverse cancers, e.g., lung cancer, endometrial cancer, and gastric cancer and was associated with dismal prognosis [34,41,44]. GLUT 3 not only plays a crucial role in glucose uptake in cancer cells but also, similarly to GLUT 1, has a remarkable correlation with the level of VEGF, which is regarded as a vital factor of cancer neovascularization. Schlößer et al. revealed the presence of GLUT 3 in 66% of the human primary gastric cancer or adenocarcinoma of the gastroesophageal junction, and higher expression of GLUT 3 was associated with advanced UICC stage of cancer. Furthermore, the expression of GLUT 3 in primary gastric cancer has a remarkable correlation with unfavorable patients' survival [34]. However, studies conducted on the human stage I non-small-cell lung carcinoma revealed that the presence of GLUT 3 in only 21% of specimens [41]. According to Tsukioka et al., GLUT 3 is also expressed in OC, and the presence of GLUT 3 was revealed in 92.8% of the human EOC specimens in their study. However, Rudlowski et al. proved the homogeneous, weak expression of GLUT 3 in the human malignant OC, and interestingly, the presence of this glucose transporter does not differ from benign lesions [53]. The current research does not prove any correlations between the expression of GLUT 3 and cancer stage or histological type. Taking into account unclear data about the expression of GLUT 3 in cancers, there is a strong need to conduct further research to confirm a precise function of GLUT 3 and whether targeting therapy by GLUT 3 inhibition may have potential clinical application in OC treatment.

4.4. A Role of GLUT 4 in OC and Other Cancers

GLUT 4 is facilitative glucose transporter predominantly expressed in insulin-dependent organs, such as brown and white adipose, skeletal and cardiac muscle [60]. GLUT 4 is found primarily in a tubulo-vesicular compartment in cells. Translocation of this transporter to the plasma membrane is mediated by insulin stimulation, which results in enhanced glucose uptake to stimulated cells [40,60]. The presence of GLUT 4 is also abundantly found in some cancerous tissues, which interestingly do not respond to insulin effects in physiological conditions, e.g., colon, lymphoid, breast, thyroid, pancreatic and gastric carcinoma [28,40,61–64]. The current state of knowledge indicates the presence and a crucial function of GLUT 4 in OC; however, precise data about these glucose transporters in carcinogenesis have been little studied, and data are still unclear [44,65]. Tsukioka et al. in revealed a higher presence of GLUT 4 in 84.4% of the human EOC specimens. Moreover, GLUT 4, similarly to GLUT 1, has a remarkable correlation with the level of VEGF, which is responsible for angiogenesis in cancer development and metastases formation [44]. This fact is confirmed by the observation that suppression of VEGFR2/AKT1/GSK3β/SOX5/GLUT 4 pathway results in attenuating tumor growth in OC [66]. Furthermore, expression of GLUT 4 varies in different histological types of OC and is significantly higher in advanced stages of OC. However, Rudlowski et al. revealed that GLUT 4 is not expressed in malignant OC [53]. There are also some studies that confirm an increased expression of GLUT 4 in malignant OC than in benign and borderline. However, in this study, the presence of GLUT 4 was higher in mucinous and clear cell adenocarcinomas, which are associated with inferior response to chemotherapy. Whereas GLUT 1 was detected to be mostly expressed in serous

adenocarcinoma. These studies indicated that there is the likelihood that overexpression of GLUT 1 and 4 is triggered by different factors in OC development [44,65]. Novel research should be conducted in order to confirm a precise function of enhanced expression of GLUT4 in OC development and provide information on whether inhibition of GLUT 4 will have beneficial effects in transporter-targeted therapies in some types of OC associated with resistance to chemotherapy because some research shows overexpression of GLUT 4 in mucinous and clear cell adenocarcinomas [65].

Understanding these facts in OC metabolism, some researchers conducted research to prove whether inhibition of GLUT 4 results in alternations in cancer cell proliferation and suppression of tumor growth. Chen et al. revealed that the impact of apatinib on OC, a tyrosine kinase inhibitor, resulted in a decrease in glucose consumption and attenuation of cancer cell proliferation in vivo in OC cells and also tumor growth suppression in vitro in mice. Apatinib was regarded in this study as a modulatory factor in glucose metabolism in OC by suppressing VEGFR2/AKT1/GSK3β/SOX5/GLUT4 pathway [66].

The vital role of glucose and its transporters—GLUTs in OC metabolism is inevitable. The current state of knowledge shows that overexpression and the role of GLUT1 are most obvious in OC, and a precise function of other facilitative glucose transporters in OC development should be assessed [34]. Resistance to chemotherapy poses a hazardous problem in OC treatment, so there is a strong need to elucidate whether a therapy targeting glycolysis pathway could have a clinical application because there are some studies that confirm its potential, beneficial effect [33,57,58,66] in Table 1.

Table 1. Facilitative glucose transporters in ovarian cancer—an overview.

Type of GLUT	Percent of OC with Overexpressed GLUT	Major Subtype of OC with Higher Expression of GLUT	Proposed Role in Dif-Ferent Cancer (Not Only in OC)	Potential Inhibitors
GLUT 1	96% [49] 98.7% [44]	- Serous adenocarcinoma [35,42,44,51,52] - Advanced stages of OC (detected only in serous adenocarcinoma) [33,44,51,53]	- Adaptation to hypoxia [49] - Basal influx of glucose, which is a source of energy [28,40] - Possibly involved in mechanism—metastasis formation [46–48] - Possibly involved in new vessel formation via VEGF [44]	- Ciglitazone—changing amount of GLUT1 in plasma membrane [57] - BAY-876—reduction of glucose utilization rate [58] - STF31 and metformin—inhibition GLUT1
GLUT 3	92.8% [44]	The current research does not prove any correlations	- Glucose reuptake [44] - Factor of neovascularization via VEGF [44]	not detected
GLUT 4	84.4% [44]	Mucinous and clear cells adenocarcinoma	Angiogenesis, metastasis formation via VEGFR2/AKT1/GSK3β/SOX5/GLUT4 pathway [66]	Apatinib–modulatory factor in VEGFR2/AKT1/GSK3β/SOX5/GLUT4 pathway [66]

4.5. A Role of MCT in OC and Other Cancers

Most cancerous cells derive energy mainly from glycolysis and have an elevated glucose utilization rate [27]. As a result of glycolysis due to the Warburg effect in cancerous cells (metabolism of glucose to lactic acid even under normoxia), a huge amount of lactic acid is produced in the malignant cells [67]. Too much lactic acid may be detrimental to cells by changing pH in intracellular fluid; thus, there is a need to efflux excessive lactate to tumor environment, vascular endothelial cells, and to an oxidative phenotype of cancer cells via monocarboxylate transporters (MCTs), mainly MCT1 and MCT 4 [68,69]. Some studies show that there is a significant overexpression of MCT1, MCT2, and MCT4 in some cancers, e.g., MCT4 in adrenocortical cancer, MCT1 and MCT 4 in cervix cancer, MCT1

and MCT 2 in brain cancer as adaptation strategy [70–73]. Monocarboxylate transporters play a predominant role in regulating pH balance in cancer cells in the case of enhanced glucose glycolysis and lactate production [67]. Moreover, the current state of knowledge indicates that lactate acts as a modulatory factor in cancerogenesis. Hence, lactate induces cytokines and growth factors secretion by macrophages, which promotes inflammation development in the tumor environment, tumor cell growth and metastasis formation [74]. Furthermore, Pinheiro et al. discovered a significant correlation between CD147 and MCT1 expressions in ovarian cancer. Interestingly, overexpressed CD147 induces the production of metalloproteinases and VEGF in cancerous tissues, and therefore, it induces cancer development and aggressiveness [67]. All of the observations mentioned above confirm that lactate metabolism contributes to cancer metastases formation. Hence, MCT's relevance in cancer development should be thoroughly elucidated.

5. Adipocytes and the Role of Lipid Transporters

Interestingly, the adipocytes play a key role in OC metastasis to the omentum (Figure 1). The experiments in mice showed that injection of OC cells in association with omental adipocytes resulted in three times bigger size of the tumor than OC alone [75]. This outcome suggests the role of tumor lipid metabolism is dependent not only on genetic and epigenetic changes but also on the bioavailability of lipids. The existence of adipose tissue in the environment of the tumor in light of current knowledge is inevitable. Taking into account the collective data describing an interaction between ovarian tumor and adipose tissue, further studies of biology and ovarian cancer cell metabolism should be conducted.

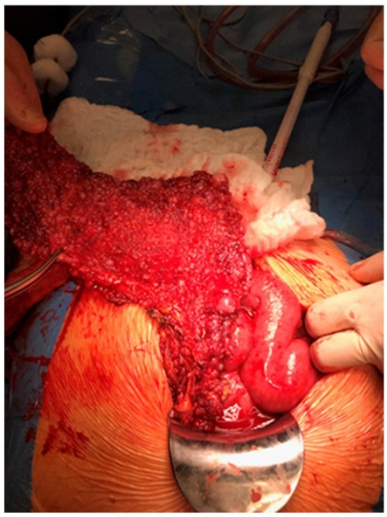

Figure 1. High-grade ovarian cancer, omental cake-like metastasis to the omentum in a 68-year old female; FIGO IIIC; 2019. University Oncology Center, Bialystok, Poland.

5.1. Fatty Acid Binding Protein 4

Despite a lack of strong evidence on the correlation between body mass and HGSC, metastatic tissues are characterized by increased expression of FABP4. The experiments on mice proved the role of FABP4 in the metastatic potential of cancer cells [76]. FABP4 is an adipocyte isoform of fatty acid-binding protein (A-FABP/FABP4/aP2). The function of FABP4 is to promote the uptake of long-chain fatty acids and to participate in lipid transport and metabolic regulation. Much research was done recently to explain the biochemical pathways and significance of this protein in cancerogenesis. There is inconsistent data

about the role of FABP4 in ovarian cancer cells. Yu et al. indicate that the increased level of this protein in ovarian cancer cells is a result of inflammation, more specifically IL17-A activity and was associated with FAs intake followed by cell growth [77]. On the contrary, Hua et al. showed that the inhibition of SRC (proto-oncogene protein tyrosine kinase Src) was associated with increased levels of FABP4 in non-small cell and renal cell carcinoma. They suggest that intracellular FABP4 plays a key role in decreasing lipid droplets, which is accompanied by the formation of ROS (reactive oxygen species) [78] (Figure 2). It is widely known that the rapid proliferation of cancer cells is associated with increased levels of ROS. To survive, the cell must activate rescue metabolic pathways to reduce the amount of ROS. The safe pathways for OC as the Warburg effect (glucose to lactate transformation observed in cancer cells) and aerobic glycolysis are induced by SRC. Therefore, treatment with SCR-inhibitor resulted in decreased tumor growth in vivo. Moreover, simultaneously treating OC with FABP4-inhibitor and SCR-inhibitor showed additive growth suppression, the opposite effect they expected. The confirmation of the inhibitory effect of FABP4 suppression on the growth of ovarian cancer cells is the work described by Mukherjee et al. [79]. Targeted therapy on FABP4 was performed in cancer cells grown together with primary human omental adipocytes. This resulted in increased levels of 5-hydroxymethylcytosine in DNA and decreased the number of clone formation and gene signatures associated with ovarian cancer metastasis. This resulted in a reduction in tumor size and metastatic potential. It is worth mentioning that the FABP4 inhibitor significantly increased the sensitivity of cancer cells to carboplatin. Taken together, these studies suggest that the adaptation of ovarian cancer cells to a lipid-rich environment is accompanied by an increased concentration of FABP4. Inhibition of these lipid transporter proteins reduces the aggressiveness of ovarian cancer, its metastasis to the peritoneum and other high-fat environments. Finally, it reduces the size of the tumor. It is worth mentioning, according to Nieman et al., we do not observe an increased concentration of FABP4 in ovarian cancer cells alone, neither in those accompanying adipocyte-depleted tissues [75]. This observation indicates the key role of FABP4 in peritoneal metastases and a large association with obesity, where there is a significant increase in the number of adipocytes.

5.2. CD36

The CD 36 receptor is an 88 kDa membrane glycoprotein and is a scavenger receptor that binds to various ligands, such as apoptotic cells, thrombospondin-1 (TSP-1) and FFA. CD36 is expressed in multiple cell types, mediates the binding and cellular uptake of long-chain fatty acids, oxidized lipids and phospholipids, advanced oxidation protein products and has roles in lipid accumulation, inflammation, apoptosis and molecular adhesion [80]. CD36 mediates the absorption of fatty acids, the major nutrients for the tumor. The fatty acids are derived from tumor-associated adipocytes and, in high concentrations, promote the proliferation and metastasis of tumor cells. This fatty acids transporter is also important in the presence of tumors by accelerating tumor growth but also inhibits angiogenesis and promotes vascular apoptosis when TSP-1 binds to CD36 on the surface of the MVEC [81]. Pascual et al. described a high ability to initiate metastasis at high concentrations of CD36, which can either be caused by a high-fat diet or induced by palmitic acid in mouse models of human oral cancer. It has also been shown that the use of CD36 inhibitors virtually completely inhibits metastasis [82]. Ladanyi et al. reported in their work that OC cells co-cultured with primary human network adipocytes showed high concentrations of CD36 in the cell membrane, which increased fatty acid uptake. The use of CD36 inhibitors prevented the development of the malignant phenotype by reducing the accumulation of lipid droplets but also reducing the concentration of ROS [83]. Last, but not least, CD36 shows a higher concentration and is more prominent in peritoneal metastases of ovarian cancer than in primary ovarian cancer and normal tissue [84]. To sum up, CD36 is another fatty acid transporter whose increased concentration is observed in the presence of adipocytes. This leads to the development of a malignant type of tumor and metastases,

most often to the peritoneum. According to observations mentioned above, CD36-targeted therapy is one of the methods of treating cancer as it can both reduce the size of a tumor and prevent or inhibit the development of a malignant tumor.

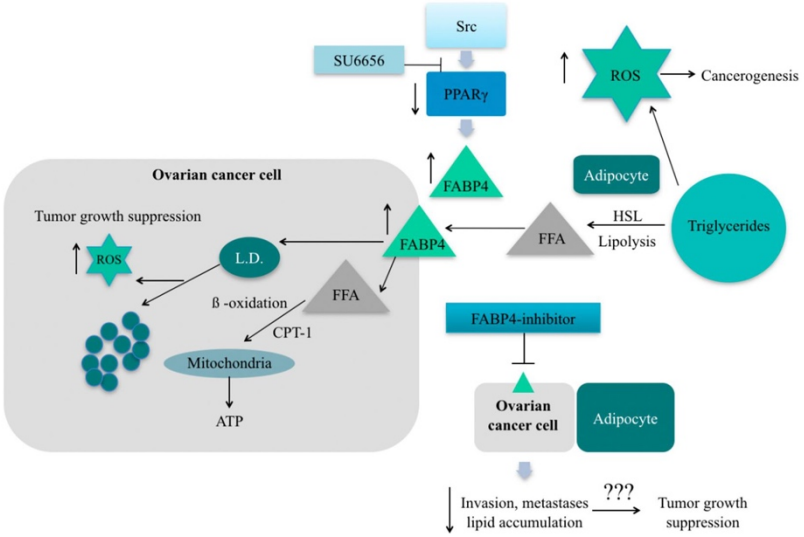

Figure 2. Proposed model of FABP4 role in the ovarian cancer cell [75,78] by Baczewska et al. SU6656—SRC-inhibitor; L.D—lipid droplets; FFA—free fatty acids; HSL—hormone-sensitive lipase.

5.3. Fatty Acid-Binding Protein 6 (FABP 6)

FABP 6 is found in adipocytes but less expressed than FABP4 and macrophages. FABP 6 is produced in the liver, then released with bile to the small gut and is involved in micelles formation. FABP 6 mediates bile acid transport in an ileal epithelium. It is commonly known that bile acids are regarded as a crucial factor initiating inflammation in colonic epithelial cells and cell death [85]. As a result of inflammation-induced in colonic cells, bile acids cause oxidative DNA damage, which is a fundamental part of malignant transformation [86,87]. Overexpression of FABP 6 is suspected to be generated by the influence of bile acids on colon cancer cells in animal models [88]. According to Ohmachi et al., FABP 6 is abundantly found in colon cancer cells compared with noncancerous tissues. Their study also revealed that there is a remarkable correlation between the size of the tumor and the expression of FABP6; more precisely, smaller tumors have an enhanced expression of FABP 6. Interestingly, levels of FABP6 expression significantly differ depending on the histological type of colorectal cancer. The current research does not prove any correlations between the expression of FABP 6 and cancer stage [87]. It is commonly known that FABP6 is present in the cell, but no less important is its elevated blood level during cancer progression. These observations confirm the recent research, which indicated FABP4 and FABP6 as potential independent biomarkers of colorectal cancer. The increased levels in the serum were associated with a higher risk of this neoplasm. Interestingly, the levels of those proteins after the surgery were significantly reduced [89].

However, there is no research that indicates any positive correlation between elevated levels of FABP6 and malignant transformation in OC. There is a strong need to conduct further research to confirm the precise function of FABP 6 in cancer development and whether there is any correlation between ovarian cancer and enhanced concentration of FABP 6 in the serum.

5.4. Role of FFA Oxidation in Ovarian Cancer Progression

The high mortality rate of ovarian cancer results from the late detection of the disease when it is in a highly advanced stage and metastasized. The spread of ovarian cancer in the body depends on the survival of the epithelial cells after their detachment and loss of nutrients due to cellular stress. Ovarian cancer cells become resistant to apoptosis as a result of fatty acid oxidation by increasing the concentration of one of the essential beta-oxidation enzymes [90]. An example of such an enzyme is carnitine palmitoyltransferase 1 (CPT1), which catalyzes the transfer of the long-chain acyl group of an acyl-CoA ester to carnitine. Studies have shown increased levels of this enzyme in OC cell lines as well as decreased survival in patients with overexpression of this enzyme. It follows that CPT1 may be a potential marker and a potential target of ovarian cancer therapy in order to limit the spread of neoplastic cells [91]. Feng et al. showed in their work that an increase in the concentration of CPT1 and Acyl-CoA dehydrogenase enzymes (ACAD enzymes) promotes epithelial ovarian cancer progression [84]. However, the function of all the enzymes involved in beta-oxidation is not fully elucidated, and data are not clear. Zhang et al. described that a low concentration of carnitine palmitoyltransferase 2 (CPT2) correlates with a decreased survival of patients with ovarian cancer and an increased frequency of metastases. This is because CPT2 suppresses the G1/S cell cycle transition as well as induces cell apoptosis. According to the authors of this study, CPT2 has the opposite effect on CPT1 and ACAD enzymes and inhibits tumor growth and metastasis dissemination [92]. It is worth mentioning that some enzymes and molecules that induce beta-oxidation can cause resistance to some anticancer drugs. A great example is collagen type XI alpha 1 (COL11A1), which is a new biomarker of cisplatin resistance in ovarian cancer. COL11A1 is both an inducer of beta fatty acid oxidation and increases the expression of proteins involved in the synthesis of fatty acids. COL11A1-induced resistance to cisplatin can be abolished by inhibiting beta fatty acid oxidation [93].

The modification of fatty acid oxidation for the therapeutic target in ovarian cancer patients has not yet been fully investigated and requires further research. The effect of individual enzymes on ovarian cancer cells varies, and the concentration of these enzymes must be increased or decreased depending on the enzyme. As a result, they seem to be a good therapeutic direction in targeted therapy and in the treatment of resistance to certain anticancer drugs.

6. Role of Adipokines

Adipose tissue is not only involved in energy storage but also plays a crucial role in hormone production, which are called adipokines [94]. Among many of them, leptin and adiponectin have the most diverse and well-known effects on our bodies [95]. Adiponectin is responsible, among others, for inducing insulin-sensitivity, antiinflammatory reactions and regulating energy metabolism [96,97]. It was shown that reduced adiponectin levels and high visceral fat could induce insulin resistance and β-cell failure [98]. There is a significant decrease in adiponectin concentrations in the serum associated with obesity [95]. The current state of knowledge indicates that adiponectins also have antitumor properties in obesity-associated cancers, such as breast, endometrial cancer, by inhibiting the ERK1/2-MAPK pathway [99]. Recent studies also indicate that adiponectins are also involved in the regulation of cancer cell invasion by inhibiting vascular endothelial growth factor, which is a potent factor initiating neovascularization [100]. Hoffman et al. proved that adiponectin suppresses ovarian cancer cell proliferation by decreasing the expression of receptors for insulin-like growth factor and estradiol [99]. Jin et al. revealed a significant decrease in adiponectin concentrations in the serum in the ovarian cancer patients compared with the control group. Moreover, their study does not prove any remarkable correlation between the stage of ovarian cancer and adiponectin level in the serum [95]. However, Tiwari et al. did not reveal any alternations in adiponectin levels in the serum in chicken models [101]. Adiponectin induces its effects on tissues by interacting with one of the receptors—adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2). There

is some research that proves a significant decrease in expression of AdipoR1 and AdipoR2 observed in ovarian cancer cell lines and, more precisely, expression of those receptors was lower in the epithelial ovarian cancer cell line compared with granulosa tumor cell line [99].

Methylation of the DNA is a process that occurs in normal and cancerous cells. The result is a change in the gene function and its final products without a change in the DNA sequence. DNA methyltransferase (DNMT1) is an enzyme that takes part in the methylation of adiponectin, the hormone that has a beneficial effect on the human body. Understanding these facts, the researchers checked whether the epigenetic targeting drugs as guadecitabin (DNMT-Inhibitor) could affect the interaction between adipocytes and OC cells. They showed that methylation is associated indirectly with metastatic cell behavior—increases cell migration and invasion, and that upregulation of suppression gene SUSD2 (Sushi Domain Containing 2) can reduce cancer cell expansion. Guadecitabin possibly changes the intracellular signaling pathways, activates the inflammatory response and indirectly prevents metastasis. These data suggest its potential use in the early-stages of Ovarian Cancer FIGO I/II, especially that authors did not observe its beneficial effect in vitro in cotreatment with alkylating Carboplatin [102].

Leptin is another type of adipokine, which also plays a broad role in normal cells and also acts as a growth factor in cancerous cells. It is involved in energy homeostasis, regulates food intake, but also exerts influence on hematopoiesis, angiogenesis and reproductive system, e.g., regulates the secretion of gonadotropin hormones [95]. The significant alternations in leptin concentration in the serum are suspected to be associated with obesity [103]. What is more, some research indicates that there is also a significant correlation between enhanced levels of leptin and developing cancer associated with obesity [95,104]. Moreover, the secretion of leptin is triggered by insulin, TNF alfa, reproductive hormones, but also by hypoxia via HIF-1, which all are involved in cancer development [104]. Leptin exerts its influence on cells by interacting with one of its receptors—LEPR and impacts diverse pathways, which promote cancerogenesis through activation of the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) [105,106]. Choi et al. revealed the expression of leptin receptors in OC and, what is more, that leptin enhances cell proliferation via the ERK1/2 pathway in OC and inhibits cell apoptosis by the inhibition of constitutive phosphorylation of p38 MAPK [107]. Furthermore, leptin is also suspected of inducing the overexpression of MMP-7 protein via ERK and JNK pathways in OC. MMP-7 induces degradation of collagen in the extracellular matrix, and as a result of that, it promotes cancer cell migration and metastasis formation in OC development [108,109]. This finding correlates with some research that proved that the inhibition of leptin-induced pathways suppresses the intraperitoneal spread of OC cells in vivo xenograft. This study revealed that PI3K/Akt/mTOR pathway induced by leptin is involved in OC peritoneal metastasis formation and interestingly showed a novel potential therapeutic target for ovarian cancer [110]. It is worth mentioning that some studies confirm that the incubation of leptin with OC cells treated with paclitaxel decreased the amount of OC cells in the G2/M phase. Paclitaxel exerts its impact on OC cells by slowing down OC cell proliferation rate by blocking the microtubule. Hence, the incubation of leptin with OC cells ameliorates the influence of paclitaxel/Taxol on cancer cell microtubules. It is worth mentioning that high levels of leptin hypothetically may have linkage with resistance to chemotherapy, but these data are unclear and need further research [111].

In summary, there is a vast majority of studies that confirm a remarkable correlation between lower expression of adiponectin, higher expression of leptin and ovarian cancer. However, there is a strong need to conduct further research to confirm the precise function of adiponectin and leptin in ovarian cancer development or whether there is any correlation between the stage of ovarian cancer, histological type and adiponectin, leptin expression. Moreover, it is also crucial to evaluate whether therapy targeting adiponectin and leptin expression will have clinical application in OC treatment.

7. Conclusions

Ovarian Cancer is a nonhomogenous disease, and its pathogenesis is still being explored. Among different biochemical phenomena, the research of the lipid and glucose pathways seems to be worth focusing on. Since the link between the expression of fatty acid and glucose transporters in the development and progression of ovarian cancer is widely investigated, many original works proved the role of adipose tissue in tumor growth and metastasis. Obesity and, more precisely, biochemical effects of the coexistence of adipocytes and ovarian cancer cells might be the key to understanding the pathogenesis of neoplastic disease progression. It is indisputable that obesity and its consequences, such as inflammation and ROS production, promote oncogenesis. Nevertheless, due to the rarity of ovarian cancer disease and ambiguous results, well-designed multicenter studies should be carried out to evaluate the precise OC risk factors.

There is a need to conduct further research to investigate new anticancer agents and assess the effects of targeted therapy on fatty acid transporters and glucose transporters. Some results of previous studies were promising because the use of particular inhibitors resulted in a decline in tumor size and a significant reduction in metastasis, mainly to the peritoneum.

Moreover, it is worth pointing out that some of FABPs could be used as potential biomarkers; however, this aspect in OC patients has not been proven yet.

Author Contributions: Conceptualization, M.B. and J.D.; methodology, M.B.; writing—original draft preparation, M.B., K.B., A.K.; writing—review and editing, M.B., K.B., A.K., J.D.; supervision, P.G., P.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

Abbreviation	Definition
OC/OvCa	Ovarian cancer
EOC	Epithelial ovarian cancer
HGSC	High-grade serous carcinoma
FABP	Fatty acid-binding protein
GLUT	Glucose transporter
MCTs	Monocarboxylate transporters
MRI	Magnetic resonance imagining
CT	Computed tomography
VEGF	Vascular epithelial growth factor
PARP-inhibitor	Poly (ADP-ribose) polymerase inhibitor
FFA	Free fatty acids
ROS	Reactive oxygen species
TSP-1	Thrombospondin-1
DNMT1	DNA methyltransferase 1
SUSD2	Sushi domain containing 2
SRC	Proto-oncogene protein tyrosine kinase
MAPK	Mitogen-activated protein kinase
CPT	Carnitine palmitoyltransferase

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9. Publikacja nr 2.

Baczewska Marta, Supruniuk Elżbieta, Bojczuk Klaudia, Guzik Paweł, Milewska Patrycja, Konończuk Katarzyna, Dobroch Jakub, Chabowski Adrian, Knapp Paweł

Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications.

Int J Mol Sci. 2022 Aug 11;23(16):8968.
IF=5,6 MEiN=140pkt.

Białystok, 2023

International Journal of
Molecular Sciences

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Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications

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Abstract: Ovarian cancer is a non-homogenous malignancy. High-grade serous carcinoma (HGSC) is the most common subtype, and its drug resistance mechanisms remain unclear. Despite the advantages of modern pharmacotherapy, high-grade ovarian cancer is associated with a poor prognosis and research into targeted therapies is in progress. The aim of the study was to assess the dominant energy substrate transport mechanism in ovarian cancer cells and to verify whether genomic aberrations could predict clinical outcomes using the Cancer Genome Atlas (TCGA) dataset. Total RNA was extracted from HGSC frozen tissues, and the expression of selected genes was compared to respective controls. *GLUT1*, *FABPpm*, *MCT4* and *SNAT1* genes were significantly overexpressed in carcinomas compared with controls, while expression of *CD36/SR-B2*, *FATP1*, *FABP4*, *GLUT4*, *ASCT2* and *LPL* was decreased. No differences were found in *FATP4*, *LAT1*, *MCT1* and *FASN*. The transcript content of mitochondrial genes such as *PGC-1α*, *TFAM* and *COX4/1* was similar between groups, while the *β-HAD* level declined in ovarian cancer. Additionally, the *MCT4* level was reduced and *PGC-1α* was elevated in cancer tissue from patients with 'small' primary tumor and omental invasion accompanied by ascites as compared to patients that exhibited greater tendencies to metastasize to lymph nodes with clear omentum. Based on TCGA, higher *FABP4* and *LPL* and lower *TFAM* expression indicated poorer overall survival in patients with ovarian cancer. In conclusion, the presented data show that there is no exclusive energy substrate in HGSC. However, this study indicates the advantage of glucose and lactate transport over fatty acids, thereby suggesting potential therapeutic intervention targets to impede ovarian cancer growth.

Keywords: ovarian cancer; glucose transporters; fatty acid transporters; monocarboxylate transporter; amino acid transporters

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Citation: Baczewska, M.; Supruniuk, E.; Bojczuk, K.; Guzik, P.; Milewska, P.; Konończuk, K.; Dobroch, J.; Chabowski, A.; Knapp, P. Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications. *Int. J. Mol. Sci.* **2022**, *23*, 8968. https://doi.org/10.3390/ijms23168968

Academic Editor: Steffen H. Hauptmann

Received: 30 June 2022
Accepted: 9 August 2022
Published: 11 August 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Ovarian cancer (OC) is the seventh overall and third gynecological most common cancer in women and is still one of the major causes of mortality with a five-year overall survival rate below 45% in advanced stages [1,2]. Most patients are diagnosed at advanced stages due to non-specific symptoms at the early stage, low efficiency of the diagnostic methods and a lack of screening tests to detect the cancer [3]. Although the effects of chemotherapy in OC are initially satisfactory, most OC cases exert major therapeutic implications during treatment and pose the threat of cancer recurrence after first-line treatment [4].

OC is a non-homogenous malignancy which varies in genetic features, molecular morphology, clinical implications and therapy [5]. High-grade serous carcinoma (HGSC) is the most common subtype, accounting for over 70% of epithelial ovarian cancers (EOCs). The hallmark feature of HGSC is the diversity in the morphology of different cancer cells, depending on their intratumoral spatial localizations and the tumor microenvironment [6], leading to widely diverse drug resistance mechanisms [7].

The development of ovarian cancer requires an adequate energy supply, which is facilitated by the interactions between cancer cells and the tumor milieu [8,9]. The heterogeneous nature of OC manifests through the co-presence of cells highly dependent on glucose that shift their metabolism towards anaerobic processes even in the presence of oxygen, and ovarian cancer cells that rely on aerobic processes such as oxidative phosphorylation [10]. Glycolysis not only enables fast provision of ATP, but also generates intermediates that are further incorporated in the biosynthesis of amino acids and lipids. Free fatty acids play a crucial role in energy production, membrane synthesis in cancer cells and oncogenic signaling [11], while their bioavailability is enhanced in a neoplastic environment [12]. As part of metabolic alterations, elevated rates of fatty acid synthesis due to increased expression of various lipogenic enzymes could account for cancer progression [11]. Moreover, if phospholipids with saturated fatty acids are abundantly expressed in cancer membranes, cancer cells are less prone to respond to chemotherapy, which also highlights the role of free fatty acids in cancer physiology [13]. Amino acids are indispensable for tumor growth as they provide nitrogen for purine and pyrimidine synthesis, serve as precursors in protein and glutathione biosynthesis, and are involved in a vast number of tumor signaling and gene expression pathways, e.g., extracellular signal-regulated protein kinase (ERK) or mammalian target of rapamycin (mTOR) cascades [14,15]. In particular, an increase in glutamine dependency in high-invasive OC preserves mitochondrial integrity by supporting reactive oxygen species (ROS) scavenging mechanisms and replenishing tricarboxylic acid cycles for energy generation [16]. Differences in predominantly used energy substrates occur even in patients with similar OC histopathological type, underlining the importance of metabolic adjustments in cancer progression [17]. According to The Cancer Genome Atlas, HGSC can be classified into immunoreactive, proliferative, differentiated, and mesenchymal cell types based on RNA sequencing and microarray data analysis [18]. Fatty acid, glucose and amino acid transporters contribute to the development, growth and metastasizing of OC, and thus become potential targets for energy metabolism-based molecular and novel cancer therapy in OC patients.

A substantial correlation between metabolic disturbances occurring in obesity and the development of human cancers was reported in both animal models and clinical trials [19–21]. Obesity triggers a wide range of inflammatory effects, which induce a vast number of biological consequences, such as ROS and cytokine production and other mechanisms, by altering the insulin/IGF-1 axis or production of steroid hormone, hence promoting oncogenesis [22]. Although OC is not suspected to be an obesity-related cancer, some studies revealed a link between enhanced body mass index (BMI) and the likelihood of developing OC, although these findings were not confirmed by other studies [23–25]. It is known that the biochemical interplay between adipocytes and OC cells might implicate invasive properties in OC and mediate carboplatin resistance. Moreover, advanced or recurrent OCs have the propensity to metastasize to adipose-rich tissue such as the omentum [26].

A large body of evidence suggests that targeting energy substrate transport in poor prognostic patients with metabolically aggressive OC may be a plausible opportunity to enhance patients' survival by introducing novel treatment schemes into conventional treatment. Therefore, the aim of this study was to assess metabolic and anthropometric factors in OC and their association with the level of expression of diverse energy substrate transporters in OC.

2. Results

2.1. Patient Characteristics

General characteristics of the control and study groups are presented in Table 1. The study group comprised 27 patients with HGSC, of which 23 patients were diagnosed with FIGO stage III–IV. Four patients carried the BRCA 1 or BRCA 2 tumor mutation. Interestingly, during surgical procedures (cytoreductive surgeries in HGSC) we observed two subgroups. Some patients presented a 'small' primary tumor and omental invasion accompanied by ascites (in our study group, seven patients). The other group had a greater tendency to metastasize to lymph nodes and omentum was clear (three patients). FIGO classification does not distinguish patients between these features. Based on in vivo research, OC cells have an affinity for fat tissue in the omentum. One of our hypotheses is that there might be molecular subgroups of HGSC that cause this heterogeneity (Table 2).

Table 1. Study and control group characteristics. Values are presented as median and interquartile range.

	Control	Ovarian Cancer	*p*
Total	n = 14	n = 27	-
Age mean	55.72 (45.58–63.75)	63.56 (57.31–70.86)	0.06
BMI (kg/m^2)	26.67 (24.92–28.72)	27.89 (24.85–33.53)	0.32
Overweight/obese	n = 9	n = 19	-
Ca125 (U/mL)	16 (10.6–26.0)	503.00 (267.00–1478.00)	<0.00001
PLT (x103 cells/mm^3)	219 (206–260)	350.00 (266.0–452.0)	0.00023
Fibrinogen (mg/dl)	317 (288–356)	453.0 (373.0–522.0)	0.0014
Serum potassium (mEq/L)	4.06 (4.0–4.3)	4.72 (4.39–5.10)	0.0016
TSH (μU/mL)	1.33 (1.18–1.6)	1.79 (1.32–2.43)	0.23
SBP (mmHg)	131 (124–149)	132 (130–145)	0.40
DBP (mmHg)	87 (83–90)	86 (73–92)	0.65
Primary tumor velocity [1]	-	109.9 (64.11–276.32)	-
Time of hospitalization (day)	4.0 (3.0–5.0)	9.5 (7.0–14.0)	0.000024

[1] Calculated using the formula $\pi/6 \times$ length $\times$ width $\times$ height. Abbreviations: BMI, body mass index; Ca125, cancer antigen 125; DBP, diastolic blood pressure; PLT, platelet count; SBP, systolic blood pressure; TSH, thyroid stimulating hormone.

Table 2. Histopathological characteristics of study group.

	n
Total	27
FIGO I	2
FIGO II	2
FIGO III	20
FIGO IV	3
BRCA 1/2 mutation	4
p53	14/19 [1]
Wilms tumor gene product (WT1)	13/15
p16	1/2
Vimentin	0/6
Estrogen receptors (ERs)	5/12
Progesterone receptors (PRs)	2/5
Nodal invasion	15/27
Omentum 'omental-cake' [2]	12/27
Nodal invasion > omental invasion [3]	3
Nodal invasion < omental invasion [4]	7
Cancer cells in peritoneal fluid	14

[1] x/y; x: number of positive samples, y: number of checked samples, if not all from study group. [2] 'Omental cake' is a specific term used to describe this serious peritoneal disease with a mass-like feature [3] Number of lymph nodes involved >50% and omentum clear [4] Number of lymph nodes involved <50% and 'omental cake'

2.2. Energy Substrate Transporters and Metabolism-Related Gene Expression

Among the tested fatty acid carriers, gene expression of cluster of differentiation 36/a scavenger receptor class B protein (*CD36/SR-B2*) and fatty acid transport protein 1 (*FATP1*) was lower in OC compared to controls (−37% and −62%, respectively). The mRNA level of membrane associated fatty acid binding protein (*FABPpm*) was upregulated (+89%), while *FATP4* expression remained relatively constant between groups. The transcript content for cytosolic fatty acid binding proteins such as *FABP4* was diminished in OC relative to control samples (−93%; Figures 1a–e and S1). There were no significant changes in *FASN* expression that would indicate enhanced de novo synthesis of fatty acids in cancer cells. The level of lipoprotein lipase (*LPL*), required for hydrolytic release of fatty acids from triacylglycerols in circulating lipoprotein particles, lowered by 78% in ovarian cancer (Figures S1 and S2).

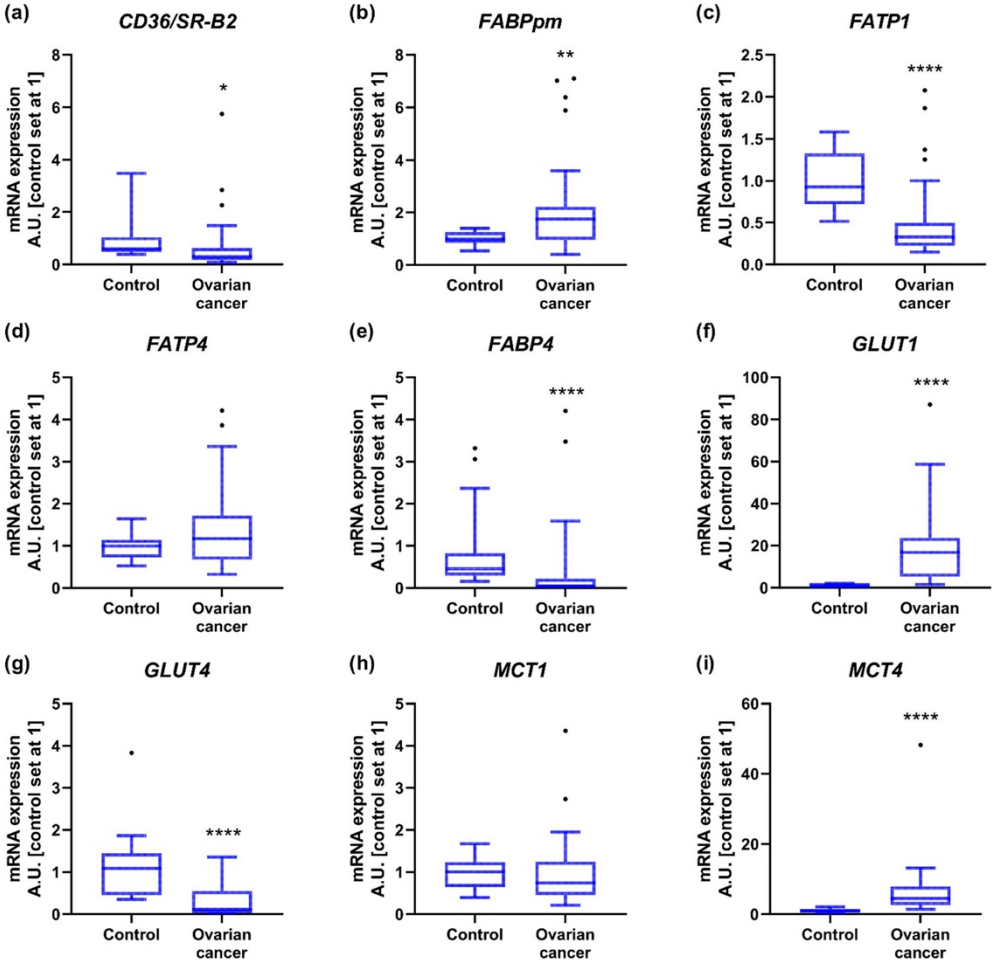

Figure 1. *Cont.*

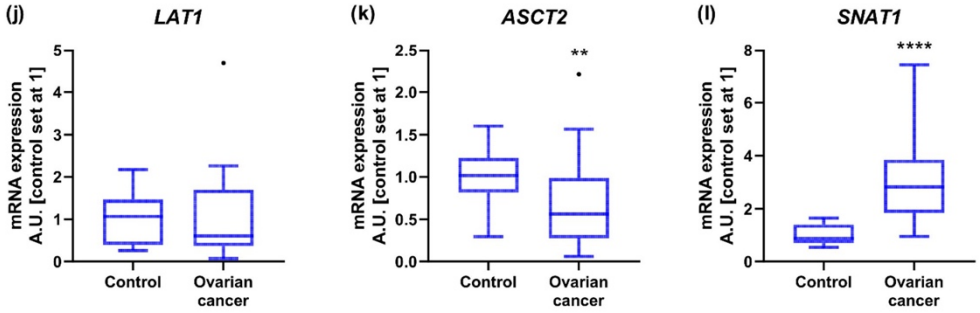

Figure 1. Transcript levels of (**a**) cluster of differentiation 36/a scavenger receptor class B protein, (**b**) membrane associated fatty acid binding protein, (**c**) fatty acid transport protein 1, (**d**) fatty acid transport protein 4, (**e**) fatty acid binding protein 4, (**f**) glucose transporter 1, (**g**) glucose transporter 4, (**h**) monocarboxylate transporter 1, (**i**) monocarboxylate transporter 4, (**j**) Na^+-independent neutral amino acid transporter, (**k**) Na^+-dependent neutral amino acid transporter and (**l**) Na^+-coupled neutral amino acid transporter 1 in ovarian control (n = 14) and cancer tissue (n = 27). Measurements were made in duplicate, and arithmetic means were used for subsequent investigation. Results are expressed in arbitrary units with control set as 1 and presented as median (min to max) value. Differences statistically significant at: * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

The expression of glucose transport proteins was enhanced in the case of *GLUT1* (+18-fold), but decreased for *GLUT4* (−90%; Figures 1f,g and S1). Regarding monocarboxylate transporters, increased transcript content was noticed for *MCT4* (+4-fold), but not for *MCT1* (Figures 1h,i and S1). Importantly, the relative mRNA expression of *GLUT1* and *MCT4* in control ovarian samples was the lowest from all the tested energy substrate transporters, and increased to the highest extent during cancer development (Figure S1).

Furthermore, we demonstrated that the mRNA level of Na^+-independent neutral amino acid transporter (*LAT1*) was comparable in both groups, while Na^+-dependent neutral amino acid transporter expression declined in OC (*ASCT2*, −44%; Figures 1j,k and S1). By contrast, the expression of Na^+-coupled neutral amino acid transporter 1 (*SNAT1*) was markedly elevated in OC (+221%; Figures 1l and S1).

Primary OC that developed metastasis was characterized by a lower expression of *LPL* (−59%) compared to tumors that had not spread to the lymph nodes. *MCT4* level was markedly diminished (−57%) in patients with 'small' primary tumor and omental invasion accompanied by ascites compared to cancer tissue from patients with greater tendency to metastasize to lymph nodes with clear omentum. Regarding other genes, we did not notice significant differences in their levels with respect to the FIGO stage as well as the presence of lymphatic node invasion or 'omental-cake' (Table A1).

2.3. Mitochondrial Gene Expression

In the next step, we verified the expression of several mitochondrial genes and observed a significant decline in *β-HAD* level in OC (−60%) compared to controls. We did not notice considerable differences in the transcript content for *PGC-1α*, *TFAM* and *COX4/1* in cancer tissue (Figure 2 and Figure S1). The expression of these genes did not differ with respect to the FIGO stage as well as the presence or absence of lymphatic node invasion or omentum 'omental-cake'. Nonetheless, *PGC-1α* level was enhanced (+14-fold) in patients with 'small' primary tumor and omental invasion accompanied by ascites, compared to cancer tissue from patients characterized by a greater tendency to metastasize to lymph nodes with clear omentum (Table A1).

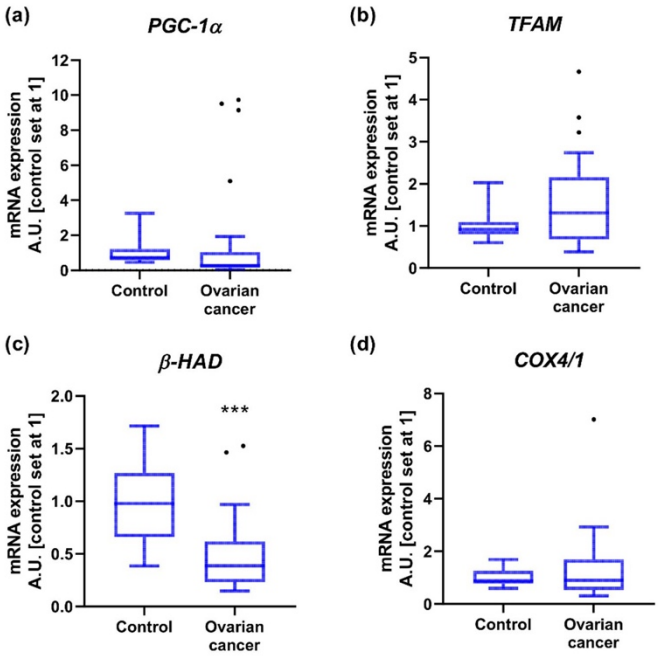

Figure 2. Transcript levels of (**a**) peroxisome proliferator-activated receptor gamma co-activator 1α, (**b**) mitochondrial transcription factor A, (**c**) acetyl-CoA acyltransferase and (**d**) cytochrome c oxidase subunit 4 isoform 1 in ovarian control (n = 14) and cancer tissue (n = 27). Measurements were made in duplicate and arithmetic means were used for subsequent investigation. Results are expressed in arbitrary units with control set as 1 and presented as median (min to max) value. Differences statistically significant at *** $p < 0.001$.

2.4. Associations of Gene Expression with BMI in Patients with Ovarian Cancer

Overweightness, defined as a BMI greater than 25 kg/m^2, was not related to changes in transcript content in the studied genes. Obesity (BMI > 30 kg/m^2), however, was associated with higher expression of *FABPpm, PGC-1α* and *FASN* in OC compared to tissue samples obtained from non-obese patients (Table 3).

Table 3. Log2-fold changes in gene expression in ovarian cancer tissue obtained from overweight (n = 9) or obese patients (n = 10) compared to lean individuals (n = 8).

Gene	Fold Change		*p* Value	
	Overweight	Obese	Overweight	Obese
CD36/SR-B2	0.9172	−0.1781	0.252	>0.999
FABPpm	0.6058	1.0061	0.329	**0.026**
FATP1	0.0376	0.552	>0.999	>0.999
FATP4	0.3567	0.8573	0.974	0.219
FABP4	2.1578	0.7444	0.51	0.288
GLUT1	−1.6832	0.3236	0.407	>0.999
GLUT4	0.737	2.214	0.779	0.094
MCT1	−0.7112	−0.3096	0.908	>0.999
MCT4	0.4707	−0.245	>0.999	0.622
LAT1	1.131	0.1321	0.856	>0.999
ASCT2	−0.2871	0.6762	0.827	0.75

Table 3. *Cont.*

Gene	Fold Change		*p* Value	
	Overweight	Obese	Overweight	Obese
SNAT1	−0.073	0.7698	>0.999	0.208
PGC-1α	1.1636	1.8047	0.089	**0.016**
TFAM	0.6391	1.0391	0.955	0.984
β-HAD	−0.575	0.2069	0.861	0.994
COX4/1	0.221	0.6918	>0.999	0.532
FASN	−0.2943	1.2679	0.948	**0.045**
LPL	0.5653	0.7144	0.888	0.201

2.5. Correlations

A correlation analysis among the values of expression of energy substrate transporters in control samples revealed negative associations between *FABP4* and *GLUT1* ($p = 0.001$, r = −0.807) as well as *LAT1* and *SNAT1* ($p = 0.037$, r = −0.569). Positive relationships involved *FABP4* with *GLUT4* ($p = 0.02$, r = 0.622), *FATP4* and *MCT1* ($p = 0.0003$, r = 0.846), *FATP4* and *MCT4* ($p = 0.005$, r = 0.723), *MCT1* and *GLUT1* ($p = 0.013$, r = 0.657), and *MCT4* and *ASCT2* ($p = 0.03$, r = 0.587). In cancer samples, we observed significant correlations between *FABP4* and *FABPpm* ($p = 0.009$, r = 0.490), *FATP4* and *MCT1* ($p = 0.009$, r = 0.489), *FATP4* and *FABPpm* ($p = 0.0004$, r = 0.636), and *SNAT1* and *GLUT1* ($p = 0.005$, r = 0.527) (Figure 3).

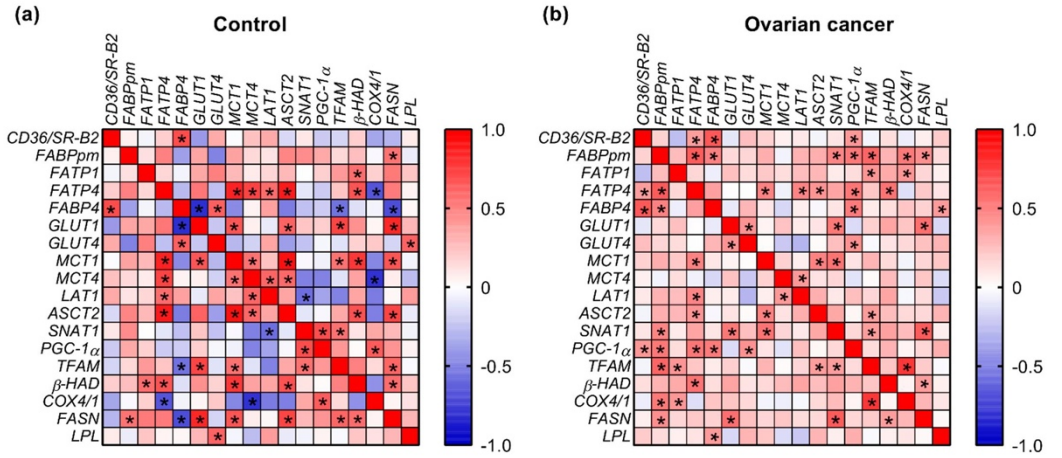

Figure 3. Heat map of correlations between the expression of energy substrate transporters and mitochondrial genes in (**a**) ovarian control and (**b**) cancer tissue. The relationships between the analyzed parameters were assayed via Spearman correlation coefficient. Correlations that were statistically significant ($p < 0.05$) are indicated with an asterisk.

The positive associations that were present in both control and cancer samples included relationships between *FABP4* and *CD36/SR-B2* (control: $p = 0.008$, r = 0.692; cancer: $p = 0.0003$, r = 0.647), *FATP4* and *LAT1* (control: $p = 0.037$, r = 0.569; cancer: $p = 0.012$, r = 0.478), *FATP4* and *ASCT2* (control: $p = 0.0001$, r = 0.868; cancer: $p = 0.03$, r = 0.418), *MCT1* and *ASCT2* (control: $p = 0.0003$, r = 0.903; cancer: $p = 0.01$, r = 0.488) as well as *MCT4* and *LAT1* (control: $p = 0.027$, r = 0.596; cancer: $p = 0.044$, r = 0.398) (Figure 3).

Additionally, in control samples we noticed a negative relationship between *TFAM* and *FABP4* ($p = 0.042$, r = −0.556), *COX4/1* and *FATP4* ($p = 0.011$, r = −0.666), *COX4/1* and *MCT4* ($p = 0.011$, r = −0.789), as well as *FASN* and *FABP4* ($p = 0.008$, r = −0.692). Positive

relationships were observed for *β-HAD* with *FATP1* ($p = 0.038$, r = 0.565), *MCT1* ($p = 0.002$, r = 0.767) and *ASCT2* ($p = 0.01$, r = 0.675), as well as for *TFAM* with *GLUT1* ($p = 0.005$, r = 0.719), *MCT1* ($p = 0.044$, r = 0.552) and *SNAT1* ($p = 0.042$, r = 0.556). In OC, there were positive relationships between *PGC-1α* and *CD36/SR-B2* ($p = 0.035$, r = 0.407), *FABPpm* ($p = 0.005$, r = 0.526), *FATP4* ($p = 0.004$, r = 0.536), *FABP4* ($p = 0.006$, r = 0.517) and *GLUT4* ($p = 0.025$, r = 0.431). Correlations common to control and OC were between *β-HAD* and *FATP4* (control: $p = 0.011$, r = 0.666; cancer: $p = 0.003$, r = 0.546) and *FASN* and *GLUT1* (control: $p = 0.001$, r = 0.824; cancer: $p = 0.002$, r = 0.563; Figure 3).

Moreover, positive correlations were found between the expression of *FABPpm* and BMI, *GLUT1* and plasma glucose concentration, and *LAT1* and tumor volume (Figure S1).

2.6. Genetic Alterations in Metabolism-Related Genes Based on TCGA and GTEx Datasets

In the next step, we verified whether our results correspond with data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects, which provide comprehensive transcriptomic data in a large number of patients with OC compared to normal ovarian tissue. We noticed only a few discrepancies between our data and TCGA data; namely, in a large OC cohort there were no alterations in *CD36/SR-B2*, *ASCT2* and *β-HAD* level (a decrease in our study) and *FABPpm* (an increase in our study). Additionally, while we did not notice alterations in *FASN* expression, in the TCGA-OC cohort this gene expression was elevated (Figure 4).

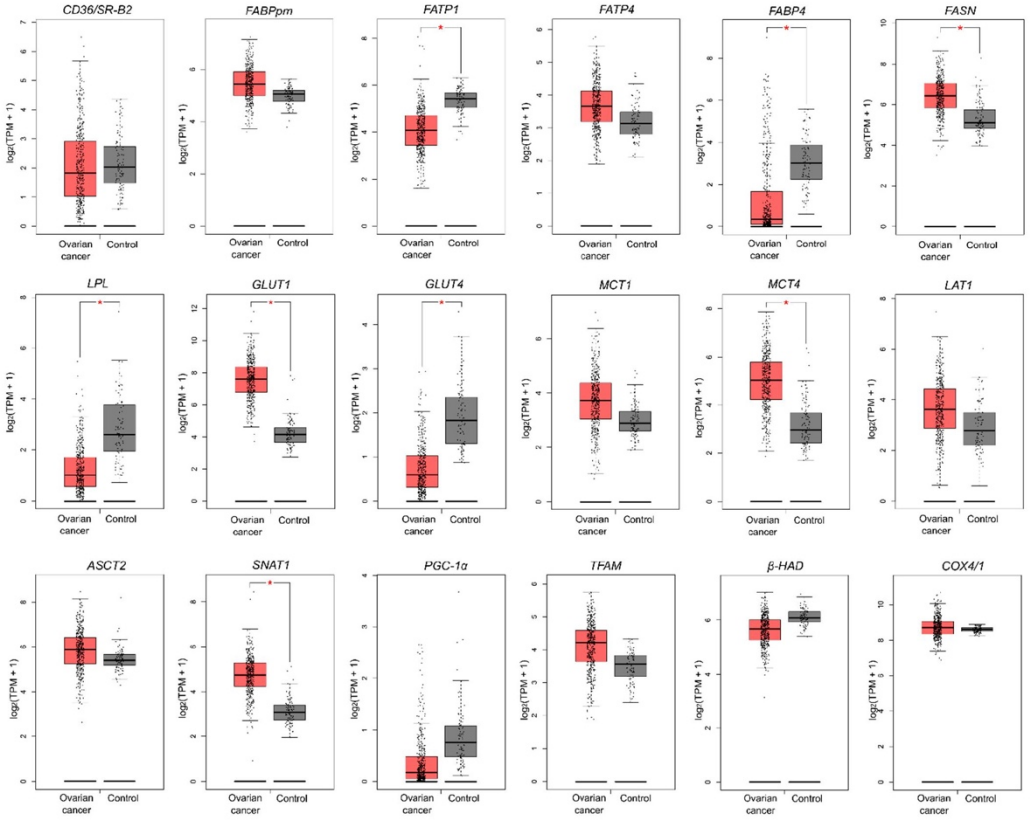

Figure 4. The transcription level of metabolism-related genes that significantly differed between

ovarian cancer (n = 426) and control tissue (n = 88). Data are based on the TCGA cancer cohort and GTEx control samples. Data derive from The Gene Expression Profiling Interactive Analysis (GEPIA) database based on the TCGA-OC dataset and normal ovarian tissues (GTEx, Genotype-Tissue Expression project). Differences statistically significant at * $p < 0.05$.

2.7. Prognostic Value of the Metabolic Pathway Genes Based on TCGA Cohort

To investigate the relationship between the expression of metabolism-related genes in OC and the clinical data, we analyzed overall survival, progression-free survival and clinical stage-related level for each gene. For overall survival analysis, higher *FABP4* and *LPL* and lower *TFAM* indicated poorer prognosis (Figures 5a and S3). In the case of progression-free survival, higher expression of *FABP4*, *PGC-1α* and *COX4/1* indicated worse, while higher levels of *GLUT4* and *TFAM* correlated with better patient progression-free survival (Figures 5b and S4). Furthermore, the expression of *FABPpm*, *FABP4*, *FASN*, *GLUT1* and *TFAM* correlated with the clinical stage of OC (Figures 6 and S5).

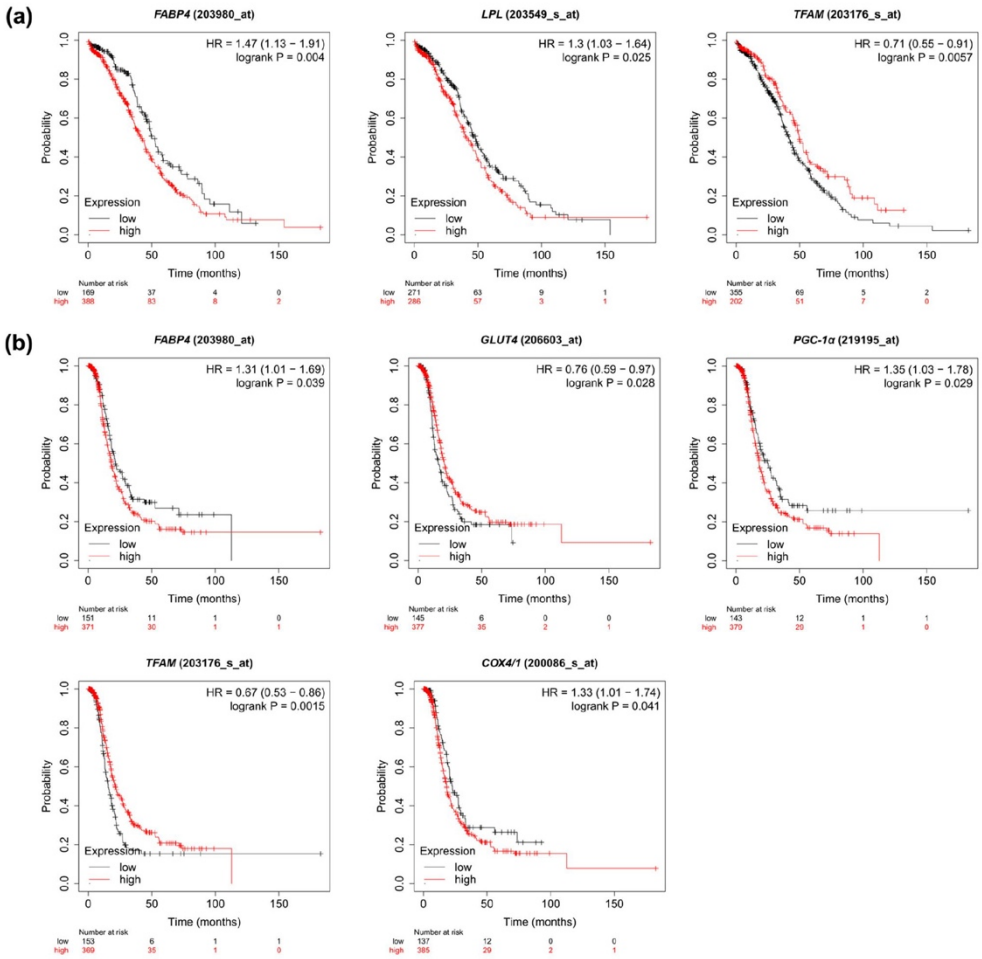

Figure 5. Kaplan–Meier curve analysis of (**a**) overall and (**b**) progression-free survival comparing high and low levels of the metabolism-associated genes.

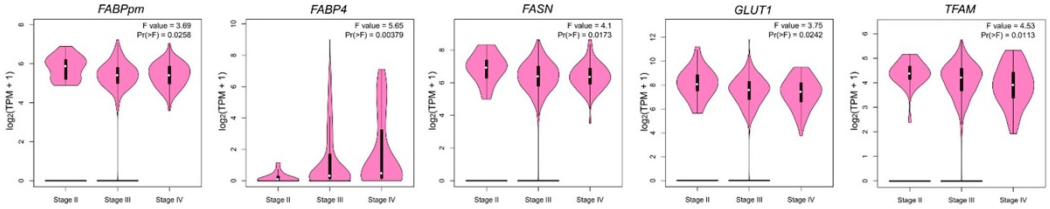

Figure 6. Violin plots representing significant correlations ($p < 0.05$) between gene expression and clinical stage based on the TCGA-OC dataset.

3. Discussion

Metabolic reprogramming has been recognized as a hallmark of tumor development because it is required to sustain the energy supply for cancer progression and metastatic dissemination. The use of nutrients depends on their availability in the tumor microenvironment and reflects the capacity for cellular genetic alterations in response to stress conditions. To explore metabolite reliance in OC, we assessed the mRNA expression of numerous proteins required for transport of fatty acids (*CD36/SR-B2*, *FABPpm*, *FATP1*, *FATP4*), glucose (*GLUT1*, *GLUT4*), monocarboxylates (*MCT1*, *MCT4*) and amino acids (*LAT1*, *ASCT2*, *SNAT1*), and also evaluated the levels of several lipid metabolism-related (*FASN*, *LPL)* and mitochondrial genes (*PGC-1α*, *TFAM*, *β-HAD* and *COX4/1*). Moreover, association analysis of the above parameters with the clinical and histological characteristics of patients was performed. Finally, the transcriptome profiles from the TCGA-OC dataset were used to associate the above-mentioned genes' expression with the clinical prognosis of OC patients.

3.1. Glucose Transporters

In our study, the *GLUT1* transcription level exerted a significantly higher expression in HGSC tissues compared to controls. This result is consistent with previous studies, which revealed that *GLUT1* mRNA and protein expression were enhanced in primary OC compared to other glucose transporters [27,28]. Moreover, concerning the stage of OC lesions, Cantuaria et al. revealed that there is a considerable correlation between the stage of OC and the expression of GLUT1, indicating that GLUT1 level is enhanced in malignant tumors compared to borderline tumors [29]. The hallmark feature of cancer cells is elevated glucose utilization by anaerobic processes such as glycolysis, even in the presence of oxygen, which is known as Warburg effect [30]. As a result of inefficient ATP production in anaerobic processes compared to oxidative phosphorylation, cancer cells are highly dependent on an enhanced influx of glucose [31]. In order to facilitate glucose uptake to maintain adequate energy supply, cancer cells enhance the expression of key factors of the glycolytic pathway, including glucose transporters (GLUTs) as well as other glycolytic enzymes [32]. The regulation of GLUT1 expression may be the reason why this glucose transporter is most prominently expressed in OC compared to other GLUTs. OC has the propensity to metastasize to the peritoneum; thus, OC cells and intraperitoneal metastases are susceptible to oxygen deprivation in the cancer microenvironment, and therefore hypoxia influences adaptive mechanisms in OC [33]. Hypoxia-inducible factor (HIF) is the main triggering factor responsible for the upregulation of GLUT1 [34]. Therefore, adaptive mechanisms to hypoxia may be a possible reason for the augmentation of GLUT1 expression in OC. In addition, genetic alterations such as P53 mutation, which is a triggering factor in developing OC, also contribute to GLUT1 upregulation [32]. On the other hand, the regulation mechanism of GLUT4 expression is not dependent on HIF-1α but rather is triggered by insulin. In our study, the expression of *GLUT4* was decreased and there is scarce information regarding the role and regulation of GLUT4 and insulin in OC [35]. Finally, the overexpression of GLUT1 is related to high-grade and poorly differentiated tumors in OC and is associated with a detrimental malignant nature of the

cancer and a dismal prognosis [8,29]. Interestingly, the application of GLUT1 inhibitor BAY-876 in OC diminished the tumor growth by 50–71%, and decreased glucose influx and consumption in both in vitro and in vivo murine models [36]. Interestingly, ciglitazone, an antidiabetic drug, exerted an anti-proliferating effect on OC cells in vitro by altering GLUT1 abundance in the plasma membrane, thus regulating glucose utilization and mitigating the rapid growth of OC [37]. Other research revealed that the GLUT1 inhibitor STF 31 and metformin, combined with chemotherapy, increased therapy efficiency in sensitive and resistant OC [38]. In addition, targeting GLUT4 appears to have therapeutic potential to hinder the development of OC. A recent study reported that apatinib, which inhibits VEGFR2/AKT1/GSK3β/SOX5/GLUT4 pathway, decreased glucose utilization in animal models in OC and exerted an anti-proliferating effect [39].

3.2. Monocarboxylate Transporters

The enhanced expression of *MCT4* mRNA in OC observed in our study is consistent with previous data that outline the augmented expression of MCT4 under hypoxic conditions and the prognostic value of MCT4 in several human cancers, e.g., cervical cancer, osteosarcoma, prostate cancer, gliomas, bladder cancer [40–42]. MCT4 is an H^+-coupled symporter that exports an excessive amount of lactate from cancer cells and is prominently expressed in metastatic tumors in a highly hypoxic environment [43]. Due to the Warburg effect, the neoplastic microenvironment is abundant in lactate and pyruvate [44]. Recently, it was revealed that MCT4 has a higher affinity for lactate compared to pyruvate and can export lactate even to a high lactate environment (MCT1 and MCT2 do not present this feature). These characteristics indicate that the expression of MCT4 takes precedence in metastatic cancers because their cells divide extensively and produce huge amounts of lactate, leading to increased acidity in the extracellular environment. Thus, the overexpression of MCT4 is an adaptive mechanism to prevent the deleterious effects of an acidic environment and hypoxia in a neoplastic environment [43]. Furthermore, recent studies demonstrated that MCT4 expression is triggered by hypoxia and mediated by HIF-1α, while MCT1 expression is not enhanced in this case [45]. Highly hypoxic areas are usually increased in large tumor lesions, along with inadequate blood flow and ascites in the tumor milieu [6]. Therefore, HIF-1α can be a plausible factor contributing to resistance to carboplatin via alterations in cancer cell metabolism by enhanced MCT4 expression [46]. The observation mentioned above highlights that the microenvironment of OC and its concomitant hypoxia contribute to the metabolic switch that results in cancer progression and treatment implications. Moreover, MCT4 expression and activity in OC are Influenced by chaperone CD147, and both are associated with dismal overall survival in several cancers [47]. The expression of CD147 was enhanced in most ovarian tumors induced by hypoxia, wherein CD147 served as an ancillary molecule for MCT-mediated lactate transport [48]. It has been shown that increased levels of MCT1, MCT4 and chaperone CD147 contribute to the development of EOC, and were correlated with high-grade tumors and ascites but not with histological type. However, only enhanced expression of MCT4, not of MCT1 or CD147, correlated with the likelihood of OC recurrence. Additionally, augmented expression of MCT4, MCT1 and chaperone CD147 was coupled with elevated levels of MDR1–multidrug resistance marker in OC. The expression of these molecules was detected in all metastatic lesions of OC after chemotherapy, so it can be stated that these molecule-mediated processes can lead to chemotherapy resistance [49]. Therefore, the MCT4-dependent mechanisms that contribute to EOC relapse need to be elucidated, because this transporter can potentially be used in targeted therapy in drug-resistant OC. The study conducted on mice with OC revealed that elevated MCT4 concentration yielded increased mouse mortality [50]. Interestingly, enhanced expression of MCT4 was observed in tumor stroma, especially in cancer-associated fibroblasts in breast cancer, whereas MCT1 was preferentially expressed in epithelial cancer cells and contributed to the influx of lactate to cancer cells. Thus, catabolic stromal cells transport lactate to epithelial cells, which have to fulfill high requirements for energy substances [51].

Recent studies demonstrate that synergy of the Warburg effect and reverse Warburg (lactate efflux) is thought to be a basis of cancer metabolism; however, data on the alterations in expression of metabolism-related transporters in OC are limited [17]. There is compelling evidence that enhanced GLUT1 expression and MCT4 expression are strongly associated with cancer progression, while the augmented expression of these transporters may be an adaptive mechanism to increased anaerobic glycolysis even under normoxia in order to maintain an adequate energy supply to an intensely proliferating cancer cell [52]. To sum up, GLUT1 mediates glucose influx and MCT4 induces lactic acid efflux, which are interdependent processes in cancer metabolism [53]. The enhanced glycolysis and maintenance of pH equilibrium in the case of intensive lactate production are hallmark abilities that contribute to progression from in situ to invasive cancer [54]. While blocking an individual MCT has been ineffective to restrain cancer growth (i.e., cancer cells escape death using glucose and glutamine as energy sources, or alternatively overexpress the other MCT isoform), combined inhibition is cytotoxic when paralleled by loss of mitochondrial NAD^+ regenerating capacity due to suppression of glycolytic ATP production [55].

3.3. Fatty Acid Transporters

Despite the upregulation of glucose transporters and glucose-dependent metabolism in several human cancers, there is scarce data on fatty acid transporters in malignant cells. In a nutrient-deprived microenvironment (limited glucose availability) cancer cells' metabolism shifts and fatty acid β-oxidation has a prominent role in maintaining an adequate energy supply for cancer cells [56]. Due to the evolving evidence for the cooperation between OC and adipocytes in the omentum, the alterations in lipid metabolism in HGSC are regarded as crucial factors in dissemination to the peritoneum and omentum [57]. In our study, however, we noticed reductions in the level of fatty acid transporters such as *CD36/SR-B2*, *FATP1* and *FABP4*, which were associated with lower *LPL* and mitochondrial *β-HAD* levels. The upregulation of glucose-related transporters coupled with a decrease in the expression of fatty acid transporters could be the cause of malignant metabolic alteration in cancers. There is compelling evidence that FABP4 chaperone protein and CD36/SR-B2 constitute pivotal regulatory factors of fatty acid transport in human cancer and are upregulated in several human cancers, e.g., breast cancer, colon cancer, cervical cancer [58–60]. FABP4 was observed in abundance in metastatic OC, which is discrepant with our study. Recent studies indicate that adipocytes and OC cells inevitably cooperate in the lipid supply and that the fatty acid transport between adipocytes and OC cells is mediated by FABP4 [61]. Congruently, the overexpression of FABP4 was associated with cancer-related adipocytes, and FABP4 mediated the transport of lipids from adipocytes to OC cells. Moreover, the overexpression of FABP4 increased the likelihood of formation of peritoneal metastases in OC [26]. In another study, the overexpression of FABP4 was associated with an adaptive mechanism and resulted in a decrease in lipid droplet formation coupled with ROS production. However, Nieman et al. did not demonstrate in their study enhanced expression of FABP4 in OC cells incubated without adipocytes, and the expression of FABP4 was enhanced in metastases compared to primary ovarian tumors [62]. The possible explanation for this discrepancy with our results is the fact that our study was conducted on OC at primary sites but not on metastases of OC from the peritoneum, which is surrounded by a lipid-abundant microenvironment. Indeed, some researchers reported that the suppression of FABP4 diminished the ability of an enhanced 5-hydroxymethylcytosine formation in DNA to invade the high-lipid cancer milieu, leading to decreased expression of genes involved in OC dissemination and enhanced susceptibility to chemotherapy both in vitro and in vivo. Thus, there is plausible evidence that targeting fatty acid transporters can be used as an alternative therapy in OC metastases [26]. Taken together, a prominent role of FABP4 is associated with metastases in the peritoneum, which develop in a microenvironment abundant in lipids that necessitates a linkage with the bioavailability of lipids in visceral fat.

CD36/SR-B2 is involved in binding and subsequently mediating the influx of long-chain fatty acids, oxidized lipids and phospholipids to cancer cells [63]. The findings of recent studies showed that cancers with enhanced expression of CD36/SR-B2, induced by a high-fat diet or by palmitic acid in mouse models of human oral cancer, have a proclivity for metastasizing [64]. Abundant presence of CD36/SR-B2 in OC was observed with concomitant adipocytes in the microenvironment. Previous research proved that OC cell lines co-cultured with human adipocytes have enhanced expression of CD36/SR-B2, which augments fatty acid influx to cancer cells. However, other FA transporters, such as FABPpm, FATP1 and FATP4, remained unaffected. Accordingly, CD36/SR-B2 is overexpressed in peritoneal metastases in OC compared to primary OC [65], whereas our study was mostly conducted on OC at primary sites and diminished expression of *CD36/SR-B2* was detected. Nevertheless, promising results were demonstrated in animal models with the use of anti-CD36/SR-B2 antibodies, which exerted growth-inhibitory effects and considerably limited the number of metastases [64,65].

Unlike other fatty acid transporters, *FABPpm* showed increased transcript content in OC tissue compared to the control group, with a positive correlation between the expression of *FABPpm* and BMI. It is hard to interpret these data since our study is one of the first to investigate the expression of *FABPpm* mRNA in OC. Therefore, future studies are necessary to determine FABPpm's role in cancer progression.

3.4. Amino Acid Transporters

A vast number of studies have demonstrated that the expression of amino acid transporters LAT1 and ASCT2 is enhanced in tumor tissues, e.g., colorectal cancer, suggesting a crucial role of amino acid supply in fulfilling the high proliferation rate in cancer cells [66]. However, scarce information is available concerning the relationship of amino acid transporter expression with cell growth in human ovarian cancer. In our study, we showed that the level of *ASCT2* expression was decreased, while *LAT1* mRNA expression remained similar in both control and cancer groups. Previous studies demonstrated that LAT1 expression was increased in vitro in OC cell lines. However, the suppression of LAT1 did not cause a significant decrease in cell anchorage-dependent growth of both SKOV3 and IGROV1 cells in a liquid medium [67]. This finding suggests that the increase in LAT1 expression in the ovarian cell lines is not triggered by the OC cell proliferation mechanism, but rather that there are some other pathophysiological mechanisms that cause the increase in LAT1 expression in this cell line. These results also indicate that other energy substrate transporters play a more prominent role in OC cell proliferation, because the inhibition of LAT1 action does not alter the growth of OC cells. The lack of *LAT1* alterations in our study may be due to the fact that study [67] was conducted on OC cell lines, whereas ours was based on surgical specimens. Moreover, another study revealed that LAT1 shows enhanced expression in clear cell carcinoma and low presence in serous carcinoma compared to other histological types. This result is consistent with results of our study conducted on HGSC. These findings could prove that the overexpression of LAT1 is associated with certain OC types, especially clear cell carcinoma [68,69]. Furthermore, LAT1 expression was enhanced in G1 adenocarcinoma compared to G3 adenocarcinoma. These results are consistent with our studies, conducted mostly on advanced OC (FIGO III and IV in 23 out of 27 patients), which reported no differences in LAT1 mRNA expression. That study also proved the inverse correlation between LAT1 expression and p53 expression [70]. This phenomenon acknowledges that high LAT1 expression is not associated with the genetic basis of HGSC.

ASCT2 provides the compounds for tumor growth and progression but also maintains an adequate energy supply [71]. Literature results describing the relationships involving *ASCT2* mRNA expression in OC are inconsistent. Some studies reported that ASCT2 expression has a linkage with FIGO stage and a correlation with unfavorable overall survival in OC patients [72]. TCGA and other studies demonstrated no alterations in *ASCT2* mRNA expression in OC cell lines [67], while herein we observed lowered *ASCT2* levels in OC.

Unlike the above transporters, which are important for rapid amino acid exchange between the cell and its milieu to maintain amino acid homeostasis, SNAT1 is known to mediate net glutamine uptake to sustain glutaminolysis at a level that supports malignant hallmarks [73]. In line with that, we showed that primary ovarian tumors exhibited elevated *SNAT1* expression to fulfill their proliferative drive. SNAT1 level was associated with survival time and metastasis status, while silencing of the gene in human melanoma [74] and breast cancer cell lines [75] promoted senescence and diminished cell migration rate, thus indicating it as an important target in anticancer therapy. Indeed, recent in vitro studies with functional inhibition of SNAT1 [76] or glutamine deprivation [77] reduced cancer cell proliferation and migration.

3.5. Mitochondrial Genes

Mitochondrial adaptive mechanisms are centrally important for cancer cell survival in the face of environmental stresses, including hypoxia and chemotherapeutic drugs. The role of PGC-1α, a major regulator of mitochondrial biosynthesis and antioxidant activator, in OC remains controversial, since some studies reported a significant increase in PGC-1α and its target (i.e., TFAM) protein levels in OC samples relative to controls [78], which could mediate chemoresistance by reducing apoptosis [79,80]. The knockdown of PGC-1α or TFAM thereby increased sensitivity to cisplatin [80]. Contradicting these observations, other studies showed that HGSCs exhibited higher expression of PGC-1α/TFAM compared to clear cell carcinoma, which was related to better prognosis and chemosensitivity to initial platin and taxane therapy [81]. These data were also supported by in vitro experiments, wherein PGC-1α overexpression led to the apoptosis of OC cells mediated by a decreased Bcl-2/Bax ratio [82]; therefore, high expression of mitochondrial markers (TFAM and TIMM23) was considered beneficial in HGSC cell lines [83]. Although, in the TCGA cohort and in our study, there were no significant differences between *PGC-1α* expression in control and cancer tissue, this transcriptional co-activator level varied relative to BMI and cancer characteristics (Table A1 and Table S1). Therapeutic implications of these associations are still unclear, but the involvement of mitochondrial proteins in the pathogenesis of HGSC and its chemosensitivity are evident.

The overt limitation of our study was the limited number of tested samples, which could impede adequate interpretation of the energy substrate transporter expression in OC. Another obstacle is the composition of tested tissue samples. In our research, ovarian tissue contained both epithelial and stromal cells. However, OC grows from the ovarian epithelium. Thus, analysis of the discrepancies in expressed genes in OC compared to control samples may pose a threat of misinterpretation because of an abundance of ovarian stromal cells, which may differ in the expression of diverse energy substrate transporters [8].

4. Materials and Methods

4.1. Study and Control Group

The present study conforms with the guidelines delineated in the Declaration of Helsinki and was approved by the Ethics Committee at the Medical University of Bialystok (permission number APK.002.221.2021). The samples were obtained by Biobank team at Medical University of Bialystok immediately after primary tumor removal. The biobanking serves as a unique entity dedicated to the collection of patients' biological material according to the Standard Operating Procedures, which ensure the highest quality systematic biobanking, novel imaging techniques, and advanced molecular analysis for precise tumor diagnosis and therapy: The Polish MOBIT project. Scraps were precisely selected, cut into pieces that consisted of endothelial and stromal cells, snap-frozen in liquid nitrogen and thereafter stored in tanks with liquid nitrogen. In total we obtained 158 OC tissues during surgical procedures. Samples were screened for eligibility, but 119 patients met the exclusion criteria (other histological type of OC, comorbidities such as diabetes, L-thyroxine intake, hyperlipidemia, BMI > 35, other metabolic disorders). In 12 cases technical problems occurred, or the quality of the material was unsatisfactory. Ultimately,

the study cohort included 27 patients with HGSC (cases were diagnosed from 2017 to 2021). The control group included healthy ovarian tissues obtained from non-oncological patients (14 met the inclusion criteria). Additionally, we analyzed clinical parameters obtained before surgery and patient questionnaires (diet, smoking history, family history, ECOG Performance Status Scale (WHO-Zubrod score), signs and symptoms). None of the patients received any treatment (chemotherapy, radiotherapy, or hormone therapy) before surgery.

4.2. Real-Time PCR Analysis

Total RNA was extracted using the NucleoSpin RNA Plus Kit with RNase-free DNase I treatment (Ambion, Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's protocol. RNA quantity and quality measurements were performed using spectrophotometry (at an absorbance OD ratio of 260/280 and 260/230). Total RNA (1 µg) served as a template for first-strand cDNA synthesis using the EvoScript universal cDNA master kit (Roche Molecular Systems, Boston, MA, USA). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the LightCycler 96 System real-time thermal cycler with FastStart essential DNA green master (Roche Molecular Systems). The following reaction parameters were applied: 15 s denaturation at 94 °C, 30 s annealing at 60 °C for *CD36/SR-B2*, *FATP1*, *FATP4*, *FABPpm*, *FABP4*, *GLUT1*, *GLUT4*, *FASN* and *β-actin* or 61 °C for *MCT1*, *MCT4*, *LAT1*, *ASCT2*, *SNAT1*, *PGC-1α*, *TFAM*, *β-HAD*, *COX4/1* and *LPL*, followed by 30 s extension at 72 °C for 45 cycles. PCR product specificity was verified by melting curve analysis. Reactions were run in duplicates and the expression was normalized against the housekeeper gene (β-actin). Results were calculated using the relative quantification method modified by Pfaffl [84]. The primers used in the study are listed in Table 4.

Table 4. Primer sequences used for real-time PCR.

Target Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Amplicon Length [bp]
CD36/SR-B2	GGTACAGATGCAGCCTCATT	AGGCCTTGGATGGAGAACA	157
FATP1/SLC27A1	GCTAAGGCCCTGATCTTTGG	CCAAGTCTCCAGAGCAGAAC	316
FATP4/SLC27A4	TGGCGCTTCATCCGGGTCTT	CGAACGGTAGAGGCAAACAA	140
FABPpm	GAAGGCAAAGGTGCGACAGT	GCCGAACGGTAGAGGCAAA	71
FABP4	GGGCCAGGAATTTGACGAAG	AACTCTCGTGGAAGTGACGC	184
GLUT1/SLC2A1	CACCACCTCACTCCTGTTAC	CCACTTACTTCTGTCTCACTCC	123
GLUT4/SLC2A4	GACCAACTAAGGCAAAGAG	CAATAGGATGCTTGTCTTCA	183
MCT1/SLC16A1	CACCGTACAGCAACTATACG	CAATGGTCGCCTCTTGTAGA	115
MCT4/SLC16A3	ATTGGCCTGGTGCTGCTGATG	CGAGTCTGCAGGAGGCTTGTG	243
LAT1/SLC7A5	CACAGAAAGCCTGAGCTTGA	CACCTGCATGAGCTTCTGA	249
ASCT2/SLC1A5	AGCTGCTTATCCGCTTCTTCAA	AGCAGGCAGCACAGAATGTA	175
SNAT1/SLC38A1	GCTTTGGTTAAAGAGCGGG	CTGAGGGTCACGAATCGGA	151
PGC-1α	AGCCTCTTTGCCCAGATCTT	GGCAATCCGTCTTCATCCAC	241
TFAM	AGCTCAGAACCCAGATGC	CCACTCCGCCCTATAAGC	115
β-HAD	CTTGCTCCGAGAGGGAGTC	AGCTCGTAGCTGGGAGGAAC	148
COX 4/1	GGTCACGCCGATCCATATAAG	TCTGTGTGTGTACGAGCTCATGA	79
FASN	CTTCCGAGATTCCATCCTACGC	TGGCAGTCAGGCTCACAAACG	131
LPL	GAGATTTCTCTGTATGGCACC	CTGCAAATGAGACACTTTCTC	276
β-actin	AGTCGGTTGGAGCGAGCATC	GGACTTCCTGTAACAACGCATCTC	115

4.3. Public Data Mining

To screen for transcriptional metabolic dysregulation in ovarian cancer from a large patient cohort, we used TCGA RNA-sequencing data from The Gene Expression Profiling Interactive Analysis (GEPIA) database. GEPIA contains validated gene expression data from the TCGA-OC cohort and the Genotype-Tissue Expression project (GTEx) based on normal ovarian tissues [85]. The association between the mRNA expression of metabolism-related genes in TCGA and overall survival and progression-free survival in ovarian cancer patients was evaluated using Kaplan–Meier plots [86].

4.4. Statistical Analysis

Statistical analyses were performed with GraphPad Prism software version 8.0 (GraphPad Software, Inc., San Diego, CA, USA). The Shapiro–Wilk test (test for normality) and Levene tests (test for homogeneity of variances) were used to determine the application of parametric or non-parametric methods. Afterwards, Student's *t*-test or Mann–Whitney U test was used to compare the differences between the groups. For multiple comparisons, the Kruskal–Wallis test followed by Dunn's post-hoc test was applied. The relationships between the analyzed parameters were assayed with Spearman's correlation coefficient. A log-rank test was applied to compare high and low levels of metabolism-related gene expression in Kaplan–Meier curves. Statistical hypotheses were verified at the 0.05 significance level.

5. Conclusions

The successful outgrowth and aggressiveness of OC relies on cellular metabolic flexibility as a response to the microenvironmental context and nutrient availability (Figure 7). Our results suggest that the metabolism of ovarian cancer is highly reliant on glucose influx mediated by GLUT1. Moreover, given the fact that the OC environment is acidic [87], the concomitant increase in MCT4 plays a pivotal role in maintaining Ph balance. Glycolysis also provides indirect metabolic precursors for the biosynthesis of nonglucidic acids crucial in amino acid and lipid transformation [56]; therefore, it may be a principal metabolic process in cancer cells. At the same time, the upregulation of amino acid transporter *SNAT1* suggests high glutamine dependence in ovarian cancer cells. Overall, our results and TCGA data analysis reveal differences in the metabolic biology of healthy and cancer cells that indicate potential therapeutic intervention targets to impede ovarian cancer cells' proliferation and metastasis.

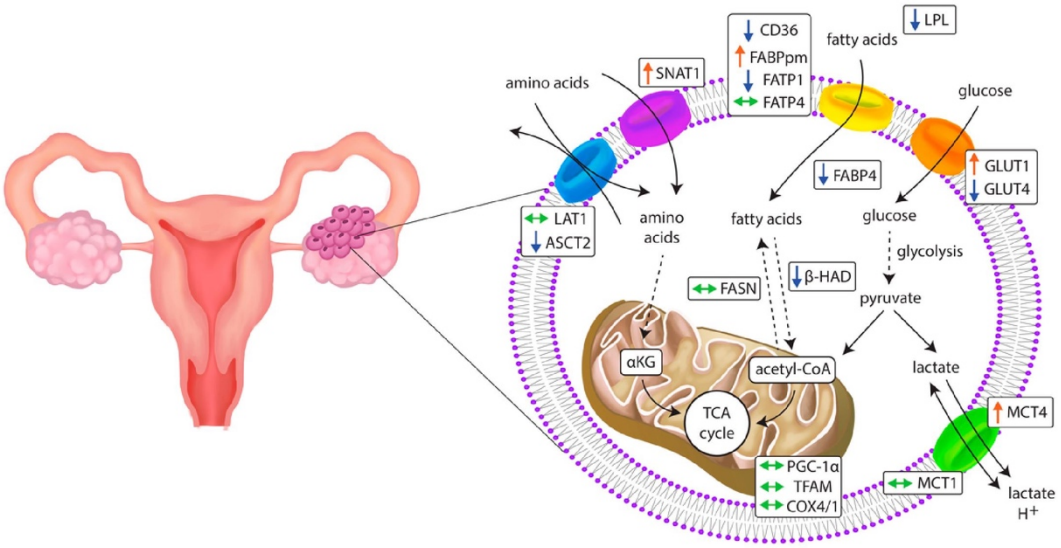

Figure 7. Changes in the expression of genes related to metabolic pathways in primary ovarian cancer tissue. ↑: increase; ↓: decrease; ↔: unchanged.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23168968/s1.

Author Contributions: Conceptualization, M.B., E.S. and P.K.; methodology, M.B. and A.C.; formal analysis, E.S. and K.K.; investigation, M.B. and E.S.; resources, M.B., P.M., J.D. and E.S.; data curation, M.B., E.S. and K.K.; writing—original draft preparation, K.B., M.B. and E.S.; writing—review and editing, P.K. and P.G.; visualization, M.B. and E.S.; supervision, P.K. and A.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Medical University of Bialystok grant number SUB/1/DN/22/007/1118.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee at the Medical University of Bialystok (permission number APK.002.221.2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The results shown here are in part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga (accessed on 1 August 2022).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

Table A1. Relative gene expression in relation to clinical–pathological parameters. The values are presented as median (min–max).

Gene	FIGO I and II vs. FIGO III and IV [1]	N0 vs. N1+N2 (Lymph Node Metastasis) [2]	Lack of vs. 'Omental-Cake'	Nodal Invasion > Omental Invasion vs. Nodal Invasion < Omental Invasion [3]
CD36/SR-B2	$p = 0.5756$ 0.61 (0.125–5.529) vs. 0.296 (0.077–2.746)	$p = 0.2773$ 0.717 (0.077–5.529) vs. 0.283 (0.135–1.427)	$p = 0.5806$ 0.482 (0.125–5.529) vs. 0.29 (0.077–2.746)	$p = 0.2371$ 0.268 (0.135–0.486) vs. 0.472 (0.17–2.746)
FABPpm	$p = 0.5756$ 1.488 (1.021–4.368) vs. 1.266 (0.273–4.859)	$p = 0.2562$ 1.49 (0.961–4.802) vs. 1.226 (0.273–4.859)	$p = 0.3473$ 1.125 (0.273–4.859) vs. 1.422 (0.594–4.802)	$p = 0.6636$ 1.294 (1.198–1.389) vs. 1.472 (0.594–4.029)
FATP1	$p = 0.9215$ 0.69 (0.345–1.828) vs. 0.642 (0.272–3.788)	$p = 0.4559$ 0.849 (0.307–3.788) vs. 0.637 (0.272–3.403)	$p = 0.4856$ 0.6 (0.307–3.788) vs. 0.868 (0.272–3.403)	$p = 0.5608$ 0.436 (0.436–0.436) vs. 0.9442 (0.2719–3.403)
FATP4	$p = 0.9737$ 1.193 (0.314–3.066) vs. 0.867 (0.234–2.811)	$p = 0.5480$ 0.98 (0.314–3.066) vs. 0.867 (0.234–2.445)	$p = 0.2773$ 0.831 (0.234–3.066) vs. 1.053 (0.429–2.811)	$p = 0.1607$ 0.665 (0.234–0.899) vs. 1.259 (0.513–2.811)
FABP4	$p \geq 0.9999$ 0.881 (0.046–2.094) vs. 0.2 (0.013–26.74)	$p = 0.2773$ 0.79 (0.046–26.74) vs. 0.161 (0.013–22.11)	$p = 0.2773$ 0.199 (0.013–10.11) vs. 0.474 (0.015–26.74)	$p = 0.3083$ 0.0472 (0.0429–1.354) vs. 1.416 (0.114–26.74)
GLUT1	$p = 0.5314$ 1.112 (0.157–2.911) vs. 0.76 (0.076–4.324)	$p = 0.1138$ 1.109 (0.157–2.911) vs. 0.519 (0.076–4.324)	$p = 0.4856$ 1.044 (0.076–4.324) vs. 0.626 (0.125–2.495)	$p = 0.698$ 0.155 (0.076–1.17) vs. 0.3119 (0.125–2.397)
GLUT4	$p = 0.2719$ 0.209 (0.155–2.327) vs. 0.141 (0.013–1.957)	$p = 0.1385$ 0.334 (0.028–2.327) vs. 0.06 (0.013–1.957)	$p = 0.8291$ 0.155 (0.013–2.327) vs. 0.155 (0.013–1.957)	$p = 0.344$ 0.034 (0.017–0.126) vs. 0.169 (0.037–1.957)

Table A1. *Cont.*

Gene	FIGO I and II vs. FIGO III and IV [1]	N0 vs. N1+N2 (Lymph Node Metastasis) [2]	Lack of vs. 'Omental-Cake'	Nodal Invasion > Omental Invasion vs. Nodal Invasion < Omental Invasion [3]
MCT1	$p = 0.5314$ 1.895 (0.295–2.387) vs. 1.025 (0.315–6.052)	$p = 0.2562$ 1.569 (0.295–6.052) vs. 0.952 (0.315–3.801)	$p = 0.4271$ 1.256 (0.295–6.052) vs. 0.93 (0.375–3.801)	$p = 0.7989$ 1.115 (0.921–1.256) vs. 0.835 (0.375–3.801)
MCT4	$p = 0.9183$ 0.812 (0.317–8.742) vs. 0.777 (0.259–2.225)	$p = 0.5267$ 0.869 (0.259–8.742) vs. 0.605 (0.316–2.225)	$p = 0.0869$ 0.995 (0.316–8.742) vs. 0.516 (0.259–1.538)	**$p = 0.0419$** 1.497 (0.357–1.609) vs. 0.481 (0.259–0.688)
LAT1	$p = 0.8693$ 0.549 (0.05–3.221) vs. 0.466 (0.139–8.853)	$p = 0.7551$ 0.416 (0.051–3.221) vs. 0.47 (0.179–8.853)	$p = 0.5164$ 0.47 (0.051–8.853) vs. 0.416 (0.139–1.300)	$p = 0.1864$ 0.47 (0.179–8.853) vs. 0.474 (0.191–1.289)
ASCT2	$p = 0.3715$ 0.86 (0.456–1.663) vs. 0.566 (0.066–2.349)	$p = 0.4559$ 0.777 (0.249–2.349) vs. 0.546 (0.066–1.278)	$p = 0.6833$ 0.566 (0.066–2.349) vs. 0.737 (0.22–1.278)	$p = 0.5431$ 0.522 (0.254–1.135) vs. 0.841 (0.296–1.153)
SNAT1	$p = 0.1910$ 1.951 (1.582–3.27) vs. 1.363 (0.516–3.996)	$p = 0.1385$ 1.94 (0.516–3.27) vs. 1.099 (0.645–3.996)	$p = 0.3473$ 1.589 (0.822–3.398) vs. 1.300 (0.516–3.996)	$p = 0.4262$ 0.994 (0.847–1.021) vs. 1.034 (0.516–3.996)
PGC-1α	$p = 0.6217$ 0.49 (0.153–1.252) vs. 0.196 (0.024–6.298)	$p = 0.7919$ 0.373 (0.024–6.298) vs. 0.181 (0.05–6.156)	$p = 0.4559$ 0.177 (0.024–6.156) vs. 0.363 (0.098–6.298)	**$p = 0.0359$** 0.024 (0.05–0.077) vs. 1.002 (0.098–6.298)
TFAM	$p = 0.2158$ 2.513 (1.643–2.950) vs. 1.516 (0.464–5.620)	$p = 0.6833$ 1.838 (0.647–3.301) vs. 1.514 (0.464–5.62)	$p = 0.8667$ 1.643 (0.513–4.308) vs. 1.838 (0.464–5.62)	$p = 0.9088$ 1.514 (1.007–4.308) vs. 1.831 (0.464–5.62)
β-HAD	$p = 0.8693$ 0.662 (0.374–2.543) vs. 0.685 (0.259–2.649)	$p = 0.3420$ 0.94 (0.298–2.543) vs. 0.62 (0.259–2.649)	$p = 0.9046$ 0.725 (0.259–2.649) vs. 0.62 (0.365–1.685)	$p = 0.1967$ 0.349 (0.259–0.865) vs. 0.873 (0.372–1.685)
COX4/1	$p = 0.1243$ 2.088 (1.021–4.29) vs. 0.894 (0.287–6.43)	$p = 0.2827$ 1.415 (0.401–4.47) vs. 0.894 (0.287–6.43)	$p = 0.7826$ 1.021 (0.287–4.29) vs. 0.873 (0.305–6.43)	$p = 0.7839$ 0.952 (0.8–2.14) vs. 0.736 (0.305–6.43)
FASN	$p = 0.4085$ 0.576 (0.375–1.636) vs. 0.895 (0.341–7.296)	$p = 0.6483$ 0.904 (0.374–2.334) vs. 0.654 (0.341–7.296)	$p = 0.7919$ 0.822 (0.341–3.03) vs. 0.858 (0.374–7.296)	$p = 0.2619$ 0.506 (0.341–0.602) vs. 0.668 (0.414–7.296)
LPl	$p = 0.6688$ 0.541 (0.39–0.763) vs. 0.46 (0.072–4.689)	**$p = 0.0044$** 0.668 (0.348–4.689) vs. 0.276 (0.072–1.399)	$p = 0.3229$ 0.39 (0.072–4.689) vs. 0.578 (0.12–3.437)	$p = 0.1833$ 0.182 (0.072–1.06) vs. 0.748 (0.275–3.437)

[1] FIGO, International Federation of Gynecology and Obstetrics system. [2] American Joint Committee on Cancer (AJCC) TNM staging system. Spread to nearby lymph nodes, also called para-aortic lymph nodes (N). [3] Refer to Table 2.

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Supplementary Material

Energy Substrate Transporters In High-Grade Ovarian Cancer - Gene Expression And Clinical Implications

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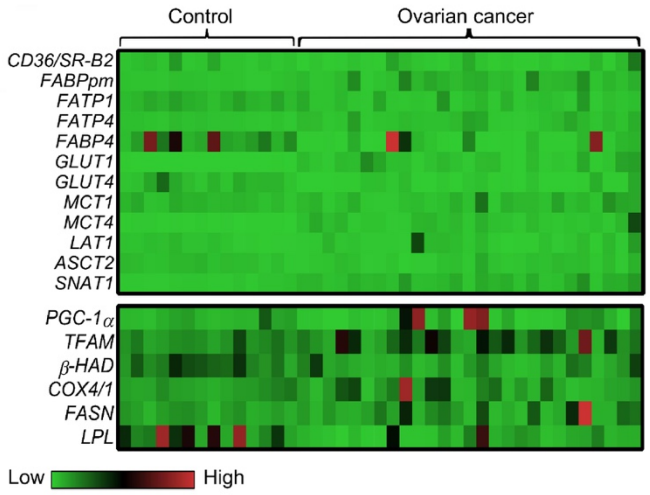

Figure S1. Heat map of relative gene expressions in control and ovarian cancer tissue.

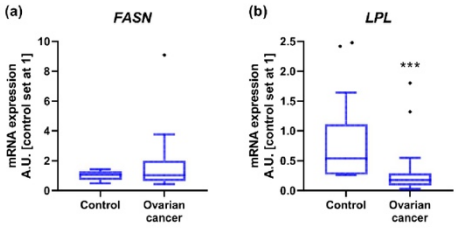

Figure S2. Transcript levels of (**a**) fatty acid synthase and (**b**) lipoprotein lipase in ovarian control (n=14) and cancer tissue (n=27). The measurements were made in duplicate, and the arithmetic means were used for subsequent investigation. The results are expressed in arbitrary units with control set as 1 and presented as median (min to max) value. *** $p < 0.001$.

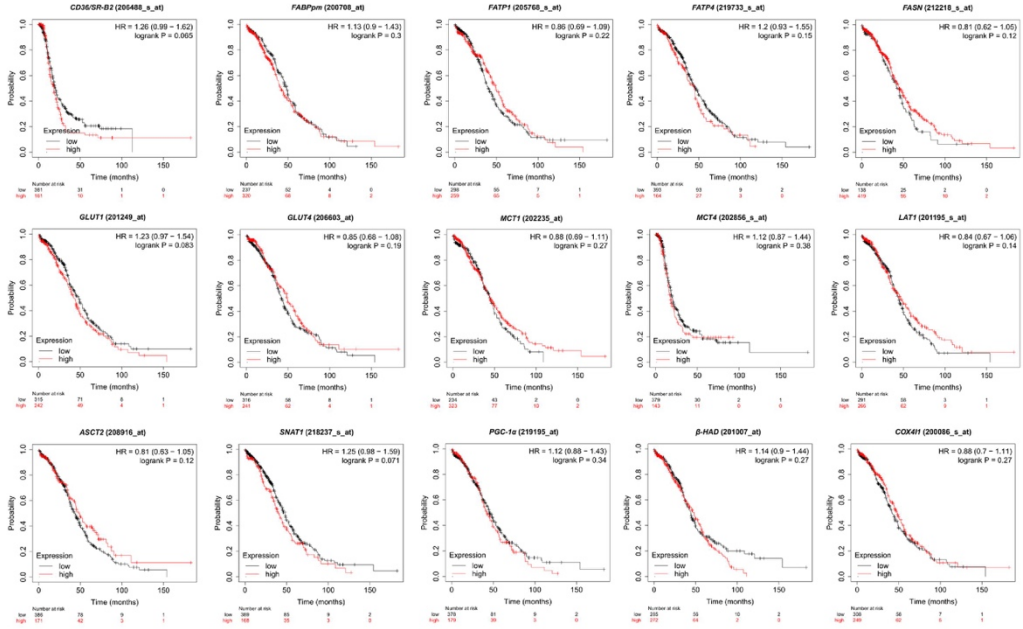

Figure S3. Kaplan-Meier curve analysis of overall survival comparing high and low levels of the metabolism-associated genes.

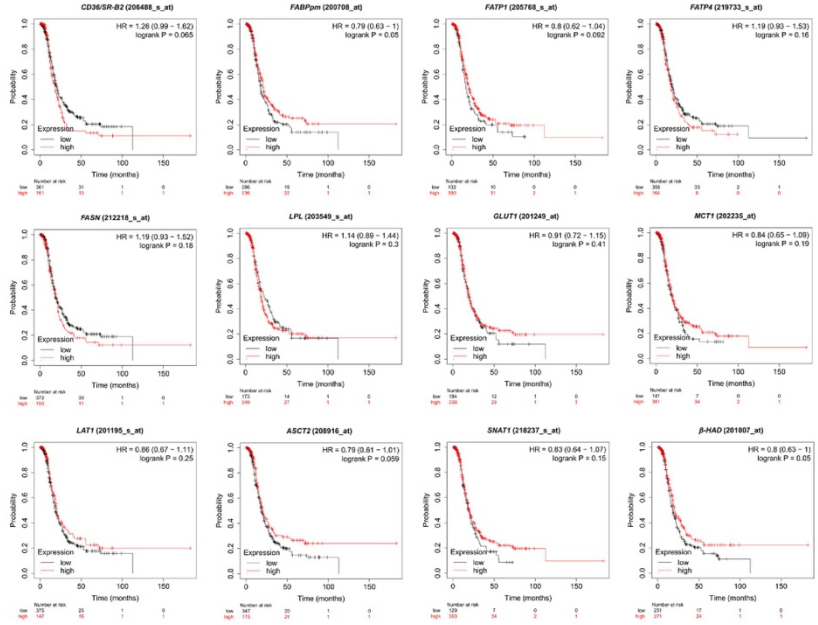

Figure S4. Kaplan-Meier curve analysis of progression-free survival comparing high and low levels of the metabolism-associated genes.

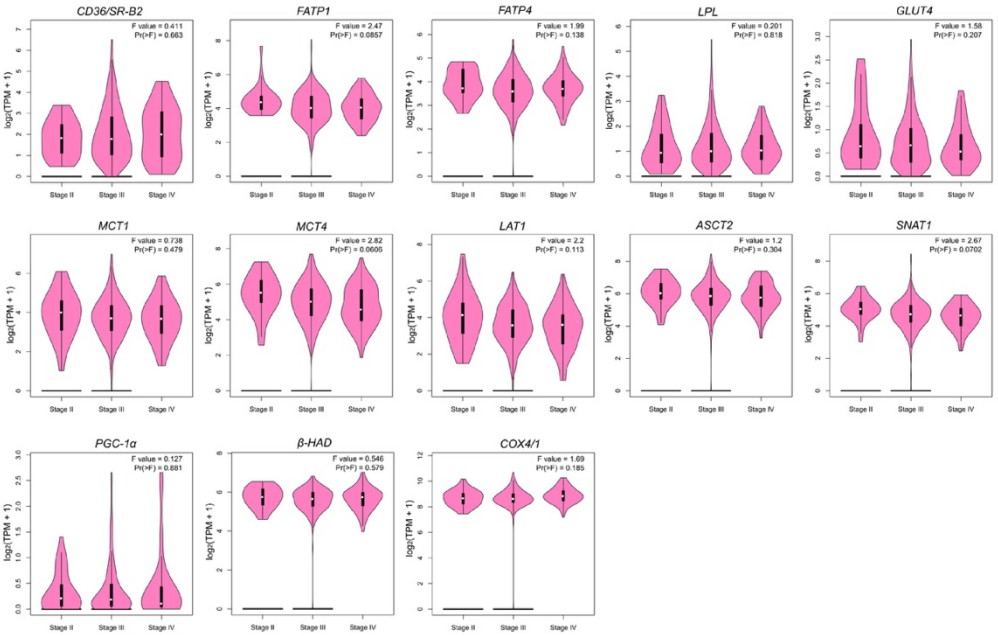

Figure S5. The violin plots depicting the correlations of metabolism-related genes with clinical stage.

Table S1. Correlations between the expression of energy substrate transporters and mitochondrial genes with BMI, plasma glucose concentration and tumor volume in ovarian cancer tissue. The relationships between the analysed parameters were assayed with Spearman correlation coefficient.

Gene	BMI		Plasma glucose level		Tumor volume	
	r	p	r	p	r	p
CD36/SR-B2	-0.0519	0.797	-0.3789	0.121	0.1374	0.494
FABPpm	0.4542	**0.017**	-0.3758	0.124	0.033	0.87
FATP1	0.0684	0.735	0.1915	0.446	0.3267	0.096
FATP4	0.2381	0.232	-0.2174	0.386	0.178	0.374
FABP4	0.235	0.238	-0.4451	0.064	-0.1197	0.552
GLUT1	-0.0226	0.911	0.5559	**0.017**	0.1206	0.549
GLUT4	0.3333	0.089	0.3509	0.153	0.1777	0.375
MCT1	0.0122	0.952	-0.1542	0.541	0.2553	0.199
MCT4	-0.2349	0.248	-0.1201	0.635	0.3084	0.125
LAT1	-0.0232	0.909	-0.4089	0.092	0.4238	**0.028**
ASCT2	0.0232	0.909	0.2039	0.417	0.2602	0.19
SNAT1	0.2418	0.224	-0.028	0.912	0.1423	0.479
PGC-1α	0.3767	0.053	-0.1864	0.459	0.2769	0.162
TFAM	0.0714	0.723	0.1128	0.656	0.0394	0.845
β-HAD	-0.0665	0.742	0.1491	0.555	0.1292	0.521
COX4/1	0.1558	0.438	-0.1097	0.665	-0.0293	0.885
FASN	0.1893	0.344	0.0714	0.778	-0.1047	0.603
LPL	0.1838	0.359	-0.1046	0.679	-0.1035	0.607

10. Streszczenie w języku polskim

Rak surowiczy jajnika o wysokim stopniu złośliwości (ang. High-grade Serous Ovarian Cancer) jest istotnym klinicznie nowotworem złośliwym, ponieważ średni odsetek 5-letnich przeżyć w zaawansowanych stadiach wynosi niespełna 50%.

Celem pracy była ocena ekspresji genów kodujących transportery substratów energetycznych, wyodrębnienie dominującego, analiza związku pomiędzy ekspresją genów a wybranymi parametrami klinicznymi i biochemicznymi oraz poszukiwanie zależności pomiędzy zmianami na poziomie genetycznym komórek nowotworowych i klinicznym przebiegiem choroby na postawie bazy danych TCGA (The Cancer Genome Atlas).

Grupa badana składała się z 27 pacjentek (operowanych w latach 2017-2021). 23 z nich prezentowało III lub IV stopień zaawansowania raka jajnika wg FIGO. Całkowite RNA ekstrahowano z zamrożonych tkanek raka surowiczego jajnika o wysokim stopniu złośliwości. Ekspresję wybranych genów na poziomie mRNA z wykorzystaniem ilościowej reakcji łańcuchowej polimerazy w czasie rzeczywistym (real-time quantative PCR) porównano z grupą kontrolną, która składała się z 14 pacjentek.

W komórkach raka jajnika zaobserwowano zwiększoną ekspresję genów *GLUT1 (*białkowego transportera glukozy typu 1*), FABPpm* (błonowego białka wiążącego kwasy tłuszczowe*), MCT4 (*transportera monokarbolsylowego*), SNAT1* (neutralnego transportera aminokwasów sprzężonego z sodem typu 1), natomiast obniżoną ekspresję *CD36/SR-B2 (*translokazy kwasów tłuszczowych*), FATP1* (białka transportującego kwasy tłuszczowe 1*), FABP4* (białka transportującego kwasy tłuszczowe 4*), GLUT4 (*białkowego transportera glukozy typu 4*), ASCT2* (transportera alaniny, seryny, cysteiny typu 2*)* i *LPL* (lipazy lipoproteinowej). Stwierdzono znaczny wzrost ekspresji *SNAT1*. Nie wyodrębniono zatem jednego preferowanego substratu energetycznego zużywanego przez tkanki nowotworowe, jednak wyniki sugerują przewagę glukozy i mleczanu nad kwasami tłuszczowymi oraz potencjalną rolę glutaminy w progresji raka jajnika.

Ekspresja analizowanych genów nie korelowała z klinicznym stopniem zaawansowania nowotworu wg klasyfikacji FIGO, inwazją węzłów chłonnych czy sieci większej. Na podstawie analizy danych TCGA, całkowity czas przeżycia był krótszy u pacjentek z wysokimi

poziomami *FABP4* i *LPL*, natomiast wysoka ekspresja *TFAM* wiązała się z lepszym rokowaniem. Ponadto dzięki zwiększonej ekspresji *MCT4*, komórki raka jajnika mogą utrzymywać sprzyjające im kwasowe środowisko, co może wpływać na ich złośliwość. Stwierdzono pozytywne korelacje pomiędzy ekspresją *FABPpm* i BMI, *GLUT*1 i stężeniem glukozy w osoczu oraz *LAT1* i objętością guza. Otyłość u pacjentek z grupy badanej wiązała się z wyższą ekspresją FABPpm, PGC-1α i FASN w porównaniu do pacjentek z BMI <30/ m2.

Podsumowując, wyniki badań laboratoryjnych oraz analiza danych z bazy TCGA potwierdzają znaczne różnice pomiędzy metabolizmem komórek prawidłowych oraz nowotworowych. Należy nadal prowadzić badania na większych grupach, aby znaleźć zastosowanie tych zjawisk w praktyce klinicznej i podejmować próby zastosowania substancji hamujących jako terapii celowanych w leczeniu nabłonkowego raka jajnika.

11. Streszczenie w języku angielskim

High-grade Serous Ovarian cancer is clinically significant gynecological neoplasm due to five-year relative survival rate of approximately 50%.

The aim of the study was to assess the dominant energy substrate transport mechanism, the analysis of the relationship between gene expression and clinical, biochemical parameters and to verify whether genomic aberrations could predict clinical outcomes using the Cancer Genome Atlas (TCGA) dataset.

The study group consisted of 27 females (surgeries were performed between 2017-2021). 23 of them presented stage III or IV according to FIGO.

Total RNA was extracted from the frozen HGSC tissues. To evaluate the expression of the mRNA, quantitative real-time polymerase chain reaction (qRT-PCR) was performed. Results were compared to control group which included 14 patients.

The increased expression of the genes *GLUT1* (glucose transporter type 1), *FABPpm* (membrane fatty acid binding protein), *MCT4* (monocarboxylate transporter), *SNAT1* (sodium-coupled neutral amino acid transporter type 1) was observed, while the expression of *CD36/SR-B2* (translocase fatty acids), *FATP1* (fatty acid transport protein 1), *FABP4* (fatty acid transport protein 4), *GLUT4* (glucose transporter type 4), *ASCT2* (alanine, serine, cysteine transporter type 2) and LPL (lipoprotein lipase) was diminished. The upregulation of *SNAT1* transcript level was found. Therefore, no single preferred energy substrate has been identified, but the results suggest the predominance of glucose and lactate over fatty acids and the potential role of glutamine in the progression of ovarian cancer.

The expression of the analyzed genes did not correlate with the clinical stage of cancer according to the FIGO classification, invasion of lymph nodes or greater omentum. Based on the analysis of TCGA data, overall survival was shorter in patients with high *FABP4* and *LPL* levels, while high *TFAM* (mitochondrial transcription factor) expression was associated with a better prognosis. Moreover, due to increased *MCT4* expression, ovarian cancer cells may maintain a favorable acidic environment, which could be related cancer aggressiveness. Positive correlations were found between *FABPpm* expression and BMI, *GLUT1* and plasma glucose concentration, and *LAT1* (neutral cellular amino acid transporter) and tumor volume. Obesity in patients from the study group was associated with higher expression of *FABPpm*, *PGC-1 α* (peroxisome proliferator-activated receptor γ coactivator 1α) and *FASN* (fatty acid synthase).- compared to patients with BMI <30/m^2.

Overall, the results and the analysis of data from the TCGA database confirm significant differences between the metabolism of healthy and ovarian cancer cells. Further research should be conducted to determine the potential clinical use of these outcomes and development of prospective therapeutic inhibiting agents to impede ovarian cancer cells' proliferation and metastasis.

12. Oświadczenia współautorów:

Obesity and Energy Substrate Transporters in Ovarian Cancer—Review	
Autorzy	**Udział w przygotowaniu publikacji**
Marta Baczewska	Opracowanie koncepcji pracy, metodologia badania, zebranie piśmiennictwa, przygotowanie rycin, analiza merytoryczna, tworzenie figur, napisanie manuskryptu, korekta manuskryptu, wysłanie manuskryptu
Klaudia Bojczuk	Napisanie manuskryptu, przygotowaniu tabeli, figur, przygotowaniu pracy do wysłania i korekcie manuskryptu.
Adrian Kołakowski	Napisanie manuskryptu, przygotowaniu tabeli, figur, przygotowaniu pracy do wysłania i korekcie manuskryptu
Jakub Dobroch	Pomoc przy napisaniu manuskryptu
Paweł Guzik	Ocena merytoryczna i korekta manuskryptu
Paweł Knapp	Nadzór merytoryczny

Białystok, 2023

Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications	
Autorzy	**Udział w przygotowaniu publikacji**
Marta Baczewska	Stworzenie koncepcji badań, tworzenie baz danych, zbieranie materiału tkankowego, udział w operacjach, podczas których były pozyskiwane tkanki, wykonywanie doświadczeń, analiza i interpretacja wyników, przygotowanie rycin i napisanie i korekta manuskryptu.
Elżbieta Supruniuk	Wykonywanie doświadczeń, analiza i interpretacja wyników, przygotowanie rycin, pomoc przy pisaniu i korekcie manuskryptu.
Klaudia Bojczuk	Tworzenie bibliografii, pomoc przy napisaniu manuskryptu i korekcie manuskryptu.
Paweł Guzik	Analiza i interpretacja wyników, pomoc przy przygotowaniu rycin i manuskryptu, korekta manuskryptu.
Patrycja Milewska	Bankowanie materiału tkankowego
Katarzyna Konończuk	Pomoc w tworzeniu baz danych i analizie statystycznej
Jakub Dobroch	Zbieranie materiału do badań
Adrian Chabowski	Metodologia, nadzór merytoryczny
Paweł Knapp	Pomoc w korekcie manuskryptu, nadzór merytoryczny

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Oświadczenie

Oświadczam, iż mój udział w przygotowaniu pracy oryginalnej pt. „Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications" opublikowanej w *International Journal of Molecular Sciences* polegał na stworzeniu koncepcji badań, tworzeniu baz danych, udziale w operacjach, podczas których były pozyskiwane tkanki, zbieraniu materiału tkankowego, wykonywaniu doświadczeń, analizie i interpretacji wyników, przygotowaniu rycin, napisaniu i korekcie manuskryptu.

Mój wkład w pracę przeglądową pt. „Obesity and Energy Substrate Transporters in Ovarian Cancer—Review" opublikowanej w *Molecules* polegał na opracowaniu koncepcji pracy oraz metodologii badania, zebraniu piśmiennictwa, przygotowaniu rycin, analizie merytorycznej, tworzeniu figur, napisaniu, korekcie i wysłaniu manuskryptu.

Białystok, 16.10.2023

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Oświadczam, iż mój udział w przygotowaniu publikacji pt. „Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications" opublikowanej w *International Journal of Molecular Science* polegał wykonywaniu doświadczeń, analizie i interpretacji wyników, przygotowaniu rycin, pomocy przy pisaniu i korekcie manuskryptu.

Jednocześnie wyrażam zgodę na włączenie wyżej wymienionej pracy do rozprawy doktorskiej lek. Marty Baczewskiej.

Elżbieta Supruniuk

Białystok, 2023

Oświadczenie

Oświadczam, iż mój udział w przygotowaniu pracy pt. „Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications" polegał na analizie i interpretacji wyników, pomocy przy przygotowaniu rycin i manuskryptu i korekcie manuskryptu. Wyrażam zgodę na włączenie pracy do rozprawy doktorskiej lek. Marty Baczewskiej.

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Oświadczam, iż mój udział w przygotowaniu pracy pt. „Obesity and Energy Substrate Transporters in Ovarian Cancer—Review" polegał na ocenie merytorycznej i korekcie manuskryptu. Wyrażam zgodę na włączenie pracy do rozprawy doktorskiej lek. Marty Baczewskiej.

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Oświadczam, iż mój udział w przygotowaniu pracy pt. „Obesity and Energy Substrate Transporters in Ovarian Cancer—Review" polegał na nadzorze merytorycznym. Wyrażam zgodę na włączenie pracy do rozprawy doktorskiej lek. Marty Baczewskiej.

Prof. dr hab. Paweł Knapp

Oświadczenie

Oświadczam, iż mój udział w przygotowaniu pracy pt. „Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications" polegał na pomocy w korekcie manuskryptu i nadzorze merytorycznym. Wyrażam zgodę na włączenie pracy do rozprawy doktorskiej lek. Marty Baczewskiej.

Prof. dr hab. Paweł Knapp

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Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji pt. „Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications" opublikowanej w *International Journal of Molecular Science* polegał na opracowaniu metodologii badań i nadzorze merytorycznym.

Jednocześnie wyrażam zgodę na włączenie wyżej wymienionej pracy do rozprawy doktorskiej lek. Marty Baczewskiej.

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Białystok, 11.10.2023 r.
miejscowość, data

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Oświadczenie

Oświadczam, iż mój udział w przygotowaniu pracy pt. „Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications" polegał na pomocy w tworzeniu baz danych i analizie statystycznej. Zgadzam się na włączenie pracy do rozprawy lek. Marty Baczewskiej.

Lek. Katarzyna Konończuk

Katarzyna Konończuk

Oświadczenie

Oświadczam, iż mój udział w przygotowaniu pracy pt. „Obesity and Energy Substrate Transporters in Ovarian Cancer—Review" polegał na napisaniu manuskryptu, przygotowaniu tabeli, figur, przygotowaniu pracy do wysłania i korekcie manuskryptu. Zgadzam się na włączenie pracy do rozprawy lek. Marty Baczewskiej.

Lek. Adrian Kołakowski

Białystok, 2023

Oświadczenie

Oświadczam, iż mój udział w przygotowaniu pracy pt. „Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications" polegał na tworzeniu bibliografii, napisaniu manuskryptu i korekcie manuskryptu. Zgadzam się na włączenie pracy do rozprawy lek. Marty Baczewskiej.

Lek. Klaudia Kołakowska (Bojczuk)

Klaudia Kołakowska

Białystok, 2023

Oświadczenie

Oświadczam, iż mój udział w przygotowaniu pracy pt. „Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications" polegał na bankowaniu materiału tkankowego. Wyrażam zgodę na włączenie pracy do rozprawy doktorskiej lek. Marty Baczewskiej.

Mgr. Patrycja Milewska

Patrycja Milewska

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Oświadczenie

Oświadczam, iż mój udział w przygotowaniu pracy pt. „Obesity and Energy Substrate Transporters in Ovarian Cancer—Review" polegał na pomocy przy napisaniu manuskryptu. Wyrażam zgodę na włączenie pracy do rozprawy doktorskiej lek. Marty Baczewskiej.

Lek. Jakub Dobroch

Oświadczenie

Oświadczam, iż mój udział w przygotowaniu pracy pt. „Energy Substrate Transporters in High-Grade Ovarian Cancer: Gene Expression and Clinical Implications" polegał na zbieraniu materiału do badań. Wyrażam zgodę na włączenie pracy do rozprawy doktorskiej lek. Marty Baczewskiej.

Lek. Jakub Dobroch

Białystok, 2023

13. Zgody Komisji Bioetycznej.

Z uwagi na użycie materiału tkankowego i danych klinicznych zbieranych w ramach „MOBIT project" za pośrednictwem Biobanku Uniwersytetu Medycznego w Białymstoku badania zawarte w niniejszej rozprawie prowadzone były na podstawie trzech zgód Komisji Bioetycznej.

1. Uchwała nr APK.002.221.2021 z dnia 23.09.2021: Projekt:„ Expression of the energy substrate transporters in High Grade Serous Ovarian Cancer".
2. Uchwała nr R-I-002/357/2014 z dnia 11.09.2014: Projekt: „Stworzenie referencyjnego modelu Diagnostyki Personalizowanej Guzów Nowotworowych w oparciu o analizę heterogenności guza z wykorzystaniem biomarkerów genomowych, transkryptomu i metabolomu oraz badań obrazowych PET/MRI jako narzędzia do wdrażania i monitorowania terapii zindywidualizowanej" akronim MOBIT.
3. Uchwała nr R-I-002/600/2019 z dnia 19.12.2019: Projekt: „Biobankowanie materiału biologicznego od pacjentów z nowotworami płuc, nowotworami przewodu pokarmowego, guzami mózgu, guzami przytarczyc, nowotworami żeńskiego układu rozrodczego".

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Białystok, 23.09.2021 r.

Uchwała nr: APK.002.221.2021

Na podstawie art. 29 ust. 2 i 14 ustawy dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty (t.j. Dz. U z 2020, poz. 514 ze zm.), Komisja Bioetyczna przy Uniwersytecie Medycznym w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP/ Guidelines for Good Clinical Practice /- **w y r a ż a z g o d ę** na prowadzenie tematu badawczego: „Expression of the energy substrate transporters in High Grade Serous Ovarian Cancer" przez prof. dr hab. Pawła Knappa wraz z zespołem badawczym z UMB.

Planowany okres realizacji od 23.09.2021 r. do 29.01.2023 r.

Przewodnicząca Komisji Bioetycznej przy UMB

prof. dr hab. Otylia Kowal-Bielecka

Pouczenie:
1. Odwołanie od uchwały komisji bioetycznej wyrażającej opinię może wnieść:
1) wnioskodawca;
2) kierownik podmiotu, w którym eksperyment medyczny ma być przeprowadzony;
3) komisja bioetyczna właściwa dla ośrodka, który ma uczestniczyć w wieloośrodkowym eksperymencie medycznym.
2. Odwołanie, o którym mowa w ust. 1, wnosi się za pośrednictwem komisji bioetycznej, która podjęła uchwałę, do Odwoławczej Komisji Bioetycznej w terminie 14 dni od dnia doręczenia uchwały wyrażającej opinię.

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Białystok, 11-09-2014

Uchwała nr: **R-I-002/357/2014**

Komisja Bioetyczna Uniwersytetu Medycznego w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP/ Guidelines for Good Clinical Practice /- **w y r a ż a z g o d ę** na prowadzenie tematu badawczego: „Stworzenie referencyjnego modelu Diagnostyki Personalizowanej Guzów Nowotworowych w oparciu o analizę heterogenności guza z wykorzystaniem biomarkerów genomowych, transkryptomu i metabolomu oraz badań obrazowych PET/MRI jako narzędzia do wdrażania monitorowania terapii indywidualizowanej. MOBIT study (Molecular Biomarkers for Individualized Therapy)" przez prof. dr hab. Jacka Niklińskiego wraz ze współpracownikami z UMB.

Przewodnicząca Komisji Bioetycznej UMB

Prof. dr hab. Elżbieta Hassmann-Poznańska

ZA ZGODNOŚĆ
Z ORYGINAŁEM

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Białystok, 19-12-2019

Uchwała nr: R-I-002/600/2019

Komisja Bioetyczna Uniwersytetu Medycznego w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP/ Guidelines for Good Clinical Practice /- **w y r a ż a z g o d ę** na prowadzenie tematu badawczego: „Biobankowanie materiału biologicznego od pacjentów z nowotworami płuc, nowotworami przewodu pokarmowego, guzami mózgu, guzami przytarczyc, nowotworami żeńskiego układu rozrodczego" przez dr hab. Joannę Reszeć wraz z zespołem badawczym z UMB.

Przewodnicząca Komisji Bioetycznej UMB

prof. dr hab. Otylia Kowal-Bielecka

14. Bibliografia

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