UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Genética

Natália Gregório Custódio

GENES DE TRANSPORTE DE MEMBRANA COMO FERRAMENTAS DE PROGNÓSTICO E PREDITORES DE RESPOSTA À QUIMIORADIOTERAPIA EM PACIENTES COM CÂNCER CERVICAL

Belo Horizonte
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Orientador: Prof. Dr. Vasco Ariston de Carvalho Azevedo

Coorientador: Prof. Dr. Wander de Jesus Jeremias

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Às quatorze horas do dia **27 de abril de 2023**, reuniu-se a Comissão Examinadora de Dissertação, indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho intitulado: **"Genes de transporte de membrana como ferramentas de prognóstico e preditores de resposta à quimioradioterapia em pacientes com câncer cervical"**, requisito para obtenção do grau de Mestre em **Genética.** Abrindo a sessão, o Presidente da Comissão, **Vasco Ariston de Carvalho Azevedo**, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra à candidata, para apresentação de seu trabalho. Seguiu-se a arguição pelos Examinadores, com a respectiva defesa da candidata. Logo após, a Comissão se reuniu, sem a presençada candidata e do público, para julgamento e expedição de resultado final. Foram atribuídas as seguintes indicações:

Prof./Pesq.	Instituição	CPF	Indicação
Vasco Ariston de Carvalho Azevedo	UFMG	283.171.225-49	APROVADA
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Maria Raquel Santos Carvalho	UFMG	349.651.730-15	APROVADA
Wander de Jesus Jeremias	UFOP	029.725.706-42	APROVADA

Pelas indicações, a candidata foi considerada: APROVADA

O resultado final foi comunicado publicamente à candidata pelo Presidente da Comissão. Nada mais havendo a tratar, o Presidente encerrou a reunião e lavrou a presente ATA, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 27 de abril de 2023.

Vasco Ariston de Carvalho Azevedo

Izinara Rosse da Cruz

Maria Raquel Santos Carvalho

Wander de Jesus Jeremias

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UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Genética

FOLHA DE APROVAÇÃO

"Genes de transporte de membrana como ferramentas de prognóstico e preditores de resposta à quimioradioterapia em pacientes com câncer cervical"

Natália Gregório Custódio

Dissertação aprovada pela banca examinadora constituída pelos Professores:

Vasco Ariston de Carvalho Azevedo
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RESUMO

Introdução: O câncer do colo do útero é o quarto câncer feminino mais comum em todo o mundo. Melhorar a sobrevida continua sendo o objetivo do tratamento no cenário clínico. Embora alguns avanços tenham sido feitos nas tecnologias de rastreamento do câncer do colo do útero, a taxa de sobrevivência e a resposta ao tratamento permanecem baixas. Os transportadores de membrana englobam muitas proteínas responsáveis por manter quantidades adequadas de moléculas dentro e fora das células. Dentre elas, destaca-se a família da ATPase e ATP sintase por seu envolvimento no processo de resistência à quimioterapia e acúmulo de platina em células inteiras. Proteínas transportadoras de soluto (SLC) também foram destacadas por desempenhar papéis críticos no movimento de agentes citotóxicos através da membrana celular neoplásica. O potencial para determinar eficácia, toxicidade e resistência à quimioterapia torna esses transportadores promissores biomarcadores preditivos para o câncer cervical. Objetivo: O presente estudo visa identificar o perfil de expressão de genes relacionados ao transporte de membrana como biomarcadores para quimioradioterapia em CC. Métodos: Seleção de células ativadas por fluorescência (FACS) foi usada para isolar células não-tronco (NCCSCs) de biópsias de câncer cervical. A biblioteca de cDNA de amostras de 21 pacientes respondedores (R) e 10 não respondedores (NR) foi construída e sequenciada usando o NextSeq® 550 Da plataforma Illumina. A análise de expressão foi realizada usando o pacote DESeq2 no programa R, considerando genes diferencialmente expressos (DEGs) para aqueles com mudança de dobra de log > 1 ou < -1 e padj ≤ 0,05. O teste estatístico de Wald e o ajuste do valor p com o método Hochberg de Benjamini também foram realizados com a mesma ferramenta. Árvores de decisão com genes expressos diferencialmente (DEGs) relacionados a transportadores de membrana foram analisadas usando o software WEKA. Resultados: Dois grandes grupos de genes com perfis de expressão diferencial opostos foram revelados. O primeiro grupo, composto principalmente pelas famílias *SLC35*, *ATP1* e *ATP5*, apresentou genes superexpressos em pacientes NR em comparação com pacientes R. O segundo grupo, representado principalmente pelas famílias *SLC25* e *ATP6*, foi superexpresso em pacientes R em comparação com pacientes NR. Os métodos de árvore de decisão definem o perfil dos genes *ATP1B3* e *SLCOB3* como capazes de classificar os pacientes em respondedores e não respondedores e para isso é necessário o corte de contagens com uma precisão de 90% no LOOCV. Os resultados sugerem que as assinaturas baseadas em *ATP1B3* e *SLCOB3* são candidatos a preditores de resposta à quimioradioterapia.

Palavras-chave: câncer cervical, SLC, ATPase, quimiorresistência

ABSTRACT

Introduction: Cervical cancer is women's fourth most common cancer worldwide. Improving survival remains the goal of the treatment in the clinical setting. Although some advances have been made in cervical cancer screening technologies, the survival rate and response to treatment remain poor. Membrane transporters encompass many proteins responsible for maintaining proper amounts of molecules inside and outside the cells. Among them, the family of ATPase and ATP synthase stands out for their involvement in the process of resistance to chemotherapy and platinum accumulation in whole cells. Solute carrier proteins (*SLC*) have also been highlighted for playing critical roles in the movement of cytotoxic agents across the neoplastic cell membrane. The potential to determine efficacy, toxicity, and resistance to chemotherapy makes these transporters promising predictive biomarkers for cervical cancer. **Objective:** The present study aims to identify the expression profile of genes related to membrane transport as biomarkers to chemoradiotherapy in CC. **Methods:** Fluorescence-activated cell sorting (FACS) was used to isolate non-stem cells (NCCSCs) from cervical cancer biopsies. The cDNA library of samples from 21 responders (R) and 10 non-responders (NR) patients were constructed and sequenced using the Illumina platform. Expression analysis was performed using the DESeq2 package in the R program, considering differentially expressed genes (DEGs) for those with log fold change > 1 or < -1 and padj ≤ 0.05. Wald's statistical test and *p*-value adjustment with Benjamini's Hochberg method were also performed with the same tool. Decision trees with differentially expressed genes (DEGs) related to membrane transporters were analyzed using WEKA software. **Results:** Two large groups of genes with opposite differential expression profiles were revealed. The first group, composed mainly of the *SLC35, ATP1, and ATP5* families, showed overexpressed genes in NR patients compared to R patients. The second group, mainly represented by the *SLC25* and *ATP6* families, was overexpressed in R patients compared to NR patients. Decision tree methods define the profile of *ATP1B3* and *SLCO1B3* genes as capable of classifying patients into responders and non-responders and cut-off of counts is necessary to this with an accuracy of 90% in LOOCV. The results suggest *ATP1B3* and *SLCO1B3* signature-based are candidate predictors of response to chemoradiotherapy.

Keywords: cervical cancer, SLC, ATPase, chemoresistance

LISTA DE FIGURAS

LISTA DE TABELAS

TABELA I Descrição das características clínicas das 31 pacientes com câncer cervical, incluindo tipo de diagnóstico, grau histológico, classificação FIGO, presença de envolvimento parametrial ou vaginal, tipo de droga utilizada no tratamento, evolução após 8 meses de tratamento e presença de metástase.

TABELA SUPLEMENTAR I Descrição do nome, função biológica e papel no mecanismo de progressão do câncer e resistência dos genes transportadores de membrana selecionados com expressão gênica diferencial. a: Informações fornecidas pelo GeneCards – o banco de dados de genes humanos.

LISTA DE ABREVIATURAS E SIGLAS

ABC *ATP-binding cassette*
ADP Difosfato de adenosina
ATP Trifosfato de adenosina
ATP sintase Adenosina trifosfato sintase
ATPase adenosina trifosfatase
BP Processo Biológico
CC Câncer do colo do útero
CCNSC Câncer não-células-tronco
cDNA Ácido Desoxirribonucleico Complementar
DEGs Genes diferencialmente expressos
DNA Ácido Desoxirribonucleico
FACS Triagem de células ativadas por fluorescência
FIGO Federação Internacional de Ginecologia e Obstetrícia
GO Ontologia Gênica
HES Hidroxietil amido
HPV Papilomavírus humano
LOOCV Validação cruzada de saída única
MF Função molecular
NKA Na+/K+ -ATPases
NR Non-responder
R Respondedora
RNA Ácido ribonucleico
SLC *Solute carrier transporter*

SUMÁRIO

1 INTRODUÇÃO

1.1 Câncer de colo de útero

O câncer cervical (CC) é um tipo de câncer que se origina no colo do útero, a parte inferior do útero que se conecta à vagina. É causada pelo crescimento anormal de células no tecido cervical e pode se espalhar para outras partes do corpo se não for tratada. Compreender a fisiologia e estadiamento do câncer cervical é essencial para o seu diagnóstico, tratamento e prognóstico (JOHNSON *et al*., 2019).

Esse tipo de câncer se desenvolve em três estágios: pré-canceroso, invasivo e metastático. No estágio pré-canceroso, células anormais estão presentes no tecido cervical, mas ainda não se espalharam além do colo do útero. Esse estágio geralmente é detectado por meio do rastreamento do câncer do colo do útero, como o teste de Papanicolau, e pode ser tratado para prevenir a progressão para câncer invasivo (BEDFORD, 2009).

No estágio invasivo, as células cancerígenas se espalharam além do colo do útero e nos tecidos circundantes. Este estágio é dividido em dois subestágios, dependendo da extensão da disseminação: estágio I, onde o câncer está confinado ao colo do útero, e estágio II, onde o câncer se espalhou para os tecidos vizinhos, mas não para o pélvico ou parede abdominal (CHAO *et al*., 2014).

No estágio metastático, as células cancerígenas se espalharam além do colo do útero e da área pélvica para outras partes do corpo, como pulmões, fígado ou ossos. Esta fase é dividida em estágio III, onde o câncer se espalhou para o terço inferior da vagina ou para a parede pélvica, e estágio IV, onde o câncer está presente em outras partes do corpo (TSIKOURAS *et al*., 2016).

O estadiamento do câncer cervical é determinado por meio de vários procedimentos diagnósticos, incluindo exame físico, biópsia cervical, tomografia computadorizada (TC), ressonância magnética (MRI) e tomografia por emissão de pósitrons (PET). A determinação correta do estágio do câncer cervical é essencial, pois direciona as possibilidades de tratamento e o prognóstico (CHI *et al*., 2017).

1.2 Epidemiologia e fatores de risco

O câncer cervical é um problema de saúde pública significativo, causando aproximadamente 311.000 mortes globalmente a cada ano. A incidência de câncer cervical varia amplamente entre os países, com as taxas mais altas encontradas em países de baixa e média renda, onde ocorrem mais de 85% dos casos (BRAY *et al*., 2018).

Os fatores de risco dessa doença são diversos, incluindo demografia, estilo de vida, comportamento sexual, genética e exposição a agentes infecciosos como o papilomavírus humano (HPV). Em particular, o HPV é um fator de risco bem estabelecido para o câncer do colo do útero, pois é encontrado em 99,7% dos casos (OLIJVE *et al*., 2018).

As mulheres com início da vida sexual em uma idade jovem, que têm múltiplos parceiros sexuais e um histórico de infecções sexualmente transmissíveis (ISTs) correm maior risco de desenvolver CC. O uso de tabaco, o uso prolongado de contraceptivos orais e imunossupressão também são importantes fatores de risco (JONES, 1995).

1.3 Prognóstico e Tratamento

O prognóstico de CC depende do estágio do câncer no momento do diagnóstico. O câncer cervical em estágio inicial (Estágios I e II) tem um prognóstico favorável, com taxas de sobrevida em 5 anos variando de 80-90% (ZHOU et al., 2020). Em contraste, o câncer do colo do útero em estágio avançado (estágios III e IV) tem um prognóstico pior, com taxas de sobrevida em 5 anos variando de 30 a 50% (CHI *et al*., 2017).

O tratamento dessa doença inclui várias modalidades, como cirurgia, radioterapia, quimioterapia e terapia direcionada e sua determinação depende, principalmente, do estágio do câncer. Procedimentos cirúrgicos como histerectomia radical (remoção do útero, colo do útero e parte da vagina) ou traquelectomia (remoção do colo do útero e tecido circundante, preservando o útero) são opções iniciais a serem consideradas. A histerectomia radical é considerada o tratamento padrão-ouro para o câncer cervical em estágio inicial e oferece a melhor chance de cura, enquanto a traquelectomia é uma opção para mulheres que desejam preservar sua fertilidade (COHEN *et al*., 2019).

O câncer do colo do útero em estágio avançado tem um prognóstico pior e pode exigir uma combinação de cirurgia, radioterapia e quimioterapia, bem como outros tratamentos, como imunoterapia ou terapia direcionada. Os medicamentos quimioterápicos usados no tratamento do câncer cervical podem incluir cisplatina, carboplatina, paclitaxel, ifosfamida, topotecano, gemcitabina e docetaxel (SCATCHARD *et al*., 2012).

1.4 Resistência a quimioterápicos em câncer de colo de útero

A resistência aos medicamentos é um grande desafio no tratamento do câncer cervical, levando ao insucesso do tratamento e à progressão da doença. Os mecanismos moleculares de resistência às drogas no câncer cervical são complexos e envolvem múltiplos fatores, incluindo alterações genéticas, mudanças nas vias de sinalização celular e presença de bombas de efluxo de drogas.

Mutações em genes-alvo de drogas, como *TP53*, podem resultar na inativação desses genes e reduzir a eficácia das drogas que têm como alvo esses genes (PETITJEAN *et al*., 2007). Em resposta ao tratamento medicamentoso, as células cancerígenas podem ativar vias alternativas de sinalização, como a via PI3K/Akt, que promove a sobrevivência celular e a resistência a medicamentos (RASCIO *et al*., 2021).

Pode-se citar também a expressão de bombas de efluxo de drogas, como os transportadores de cassete de ligação de ATP (ABC), que pode resultar no efluxo de drogas de células cancerígenas e reduzir sua eficácia (HOLOHAN *et al*., 2013).

Por fim, a presença de heterogeneidade tumoral, na qual as células cancerígenas exibem diferentes características genéticas e fenotípicas, pode resultar no surgimento de subpopulações resistentes a medicamentos e contribuir para o fracasso do tratamento (DAGOGO-JACK & SHAW, 2017).

1.5 Transportadores de membrana

1.5.1 Proteínas transportadoras de soluto (SLC)

SLC se refere a uma família de proteínas responsáveis pelo transporte de várias substâncias através das membranas celulares. Essas proteínas desempenham um papel crucial na manutenção da homeostase dentro da célula e ajudam a regular vários processos fisiológicos, como equilíbrio iônico, captação de neurotransmissores e metabolismo de drogas (HEDIGER *et al*., 2013).

A estrutura dos SLCs varia, mas eles geralmente consistem em múltiplos domínios transmembranares que formam um poro ou canal através do qual o soluto é transportado. A função dos SLCs é determinada pelo tipo específico de soluto que eles transportam e pelas características estruturais dos domínios transmembranares (PIZZAGALLI *et al*., 2020).

Os SLCs têm sido implicados no desenvolvimento e progressão do câncer, pois alterações em sua expressão podem levar a alterações nas vias de sinalização celular, equilíbrio iônico e metabolismo de drogas, o que pode contribuir para o crescimento descontrolado e a sobrevivência das células cancerígenas (AHN & NIGAM, 2009). Por exemplo, o gene *SLC22A1*, que codifica um transportador de fosfato dependente de sódio, está associada com a progressão da doença do carcinoma hepatocelular e colangiocarcinoma e uma pobre sobrevida do paciente após o tratamento com o anticancerígeno, sugerindo seu necessário papel na absorção de drogas (BROSSEAU & RAMOTAR, 2019).

Além disso, o gene *SLCO1B1*, que codifica um transportador hepático de drogas, demonstrou estar envolvido na resistência das células cancerígenas às drogas quimioterápicas (FENG *et al*., 2018). Essas descobertas destacam o importante papel que os SLCs desempenham no desenvolvimento e progressão do câncer e o potencial para direcionar essas proteínas como uma estratégia para a terapia do câncer.

1.5.2 ABC

A superfamília *ATP-binding cassette* (ABC) de transportadores de proteínas está presente em todos os organismos. Essa família de genes codifica várias proteínas que atuam como importadoras e exportadoras e facilitam o movimento de uma ampla gama de substratos, incluindo açúcares, aminoácidos, peptídeos, íons, colesterol, metabólitos e toxinas, através das membranas celulares, tanto dentro como fora da célula (BENADIBA & MAOR, 2016).

O genoma humano contém 49 genes ABC conhecidos, que são classificados em sete famílias com base em sua sequência de aminoácidos e domínios de proteínas. Esses genes desempenham várias funções, como resistência a drogas e podem ser expressos como canais, receptores e transportadores. Os membros envolvidos no efluxo de drogas não se limitam a uma família, e há 12 transportadores conhecidos por serem responsáveis pelo efluxo de drogas (SHAROM, 2008).

As proteínas ABC têm sido implicadas no desenvolvimento e progressão do câncer, pois alterações em sua função podem levar a mudanças na resistência a drogas, vias de sinalização celular e metabolismo lipídico, o que pode contribuir para o crescimento descontrolado e sobrevivência de células cancerígenas (EL-AWADY *et al*., 2017).

Várias investigações demonstraram a regulação positiva do gene *ABCB1* em espécimes clínicos, incluindo leucemia e vários tipos de câncer, como rim, cólon, mama e câncer de pulmão. Na maioria dos casos, a expressão de *ABCB1* está ligada a uma resposta reduzida à quimioterapia, indicando um mau prognóstico (ROBEY *et al*., 2010). Como resultado dessas descobertas, foram realizados ensaios clínicos para examinar a teoria *ABCB1*. A hipótese é que as drogas que inibem a função de transporte *ABCB1* podem aumentar o acúmulo de drogas e melhorar a resposta à quimioterapia, resultando em melhores resultados clínicos (ROBEY *et al*., 2018).

1.5.3 ATPases

ATPases são enzimas que hidrolisam ATP (trifosfato de adenosina), liberando energia que pode ser utilizada pelas células para diversos processos biológicos. Existem vários tipos de ATPases, sendo as V-ATPases as principais nos mamíferos (DACH & NISSEN, 2013).

As V-ATPases estão presentes em várias membranas intracelulares, incluindo lisossomos, endossomos e vesículas secretoras, bem como na membrana plasmática em células especializadas. Eles criam um ambiente ácido necessário para a degradação de proteínas por proteases dependentes de ácido, transportam pequenas moléculas e íons e conduzem a captação de neurotransmissores (RUDNICK & CLARK, 1993).

Nos endossomos essas enzimas funcionam nos processos de tráfego de membrana e estão envolvidas na endocitose mediada por receptores, no tráfego intracelular de enzimas lisossômicas e na reciclagem de receptores para a membrana plasmática (GRUENBERG & VAN DER GOOT, 2006).

A expressão de ATPases pode estar alterada em tecidos cancerosos, o que leva à manutenção de um gradiente de pH anormal entre o citosol alcalino e o ambiente extracelular ácido. Essa mudança do pH em direção à acidez no microambiente tumoral extracelular pode estimular a secreção e a ativação de proteases (SENNOUNE *et al*., 2004). Além disso, o baixo pH do ambiente extracelular pode facilitar a degradação e reestruturação da matriz extracelular por meio da ativação de enzimas proteolíticas (FAIS *et al*., 2007). Como resultado, esse processo pode contribuir para a invasão e metástase do câncer. Portanto, pode-se concluir que as ATPases podem promover a invasão e metástase do câncer (ROFSTAD *et al*., 2006).

1.5.4 ATP sintase

A ATP sintase é um sistema enzimático complexo que catalisa a síntese de ATP (trifosfato de adenosina) a partir de ADP (difosfato de adenosina) e fosfato inorgânico. Este processo é chamado de fosforilação oxidativa e é a fonte primária de produção de ATP nas mitocôndrias (JONCKHEERE *et al*., 2011).

As células cancerígenas modificam suas vias metabólicas para regular vários processos e normalmente há o aumento da captação de glicose e glicólise na maioria dos cânceres, levando à produção de ATP e ácido lático no citosol, mesmo quando as mitocôndrias são funcionais sob normóxia, uma condição conhecida como efeito Warburg. Em tumores hipóxicos, a glicólise aumenta para produzir ATP suficiente para a homeostase bioenergética (GALBER *et al*., 2020).

No efeito Warburg, a maioria das células cancerígenas depende da glicólise aeróbica, portanto, as mitocôndrias permanecem funcionais, sendo a síntese de ATP e a respiração essenciais para que a proliferação celular rápida ocorra. Estudos já demonstraram que a inibição da síntese de ATP é capaz impedir essa proliferação desordenada em diferentes linhagens celulares derivadas de câncer como glioblastoma humano e adenocarcinoma cervical (SULLIVAN *et al*. 2015).

A alteração da expressão do gene da ATP sintase tem sido relatada em vários estudos. Em carcinomas sólidos, a diminuição da expressão da subunidade β pode levar a alterações na síntese de ATP mitocondrial, enquanto níveis reduzidos da subunidade α foram encontrados em amostras de câncer de próstata e estão associados ao início precoce da doença em pacientes (FEICHTINGER *et al*. 2018). Por outro lado, a regulação positiva da subunidade α foi associada ao desenvolvimento de tumores colorretais (SETH *et al*. 2009), enquanto as subunidades α e β foram reguladas positivamente no glioblastoma e células endoteliais no microambiente tumoral (XU & LI, 2015).

1.6 Justificativa

Quimioterapia, cirurgia, radioterapia e braquiterapia são as opções de tratamento padrão para o câncer cervical. Entretanto, alguns pacientes podem desenvolver resistência a esses tratamentos, dificultando a decisão sobre o seguimento terapêutico. Os mecanismos responsáveis pelas alterações moleculares envolvidas na quimiorresistência no câncer cervical ainda não são totalmente compreendidos (JALIL *et al*., 2021).

As proteínas de transporte de membrana podem ser ferramentas preditivas e prognósticas promissoras no câncer, pois os eventos relacionados a esse transporte são essenciais na determinação do destino celular para sobrevivência, morte ou transformação maligna (ALMASI & EL HIANI, 2020). Também mantêm o equilíbrio entre toxicidade e manutenção dos quimioterápicos, controlando sua absorção e distribuição entre os tecidos. Assim, alterações no perfil de expressão de proteínas de transporte de membrana podem estar associadas à tumorigênese e/ou quimiorresistência (MITRA & ELANGOVAN, 2021).

O objetivo deste estudo é identificar possíveis genes transportadores que apresentem correlação entre seu padrão de expressão diferencial e o desenvolvimento de resistência à quimioradioterapia em pacientes com câncer cervical. Isso ajudaria a tomar decisões mais precisas e fornecer uma terapia mais eficiente.

1.7 Contextualização

Esse estudo foi realizado em parceria com o Instituto Mario Penna, local que desenvolve diversos trabalhos relacionados ao câncer, principalmente os femininos. Sendo assim, as amostras utilizadas já tinham sido sequenciadas e analisadas para outras pesquisas envolvendo tumor de colo de útero.

A partir dessas amostras, o estudo foi desenhado tendo como principal objetivo a obtenção de marcadores moleculares que facilitem a decisão da equipe médica e torne o tratamento mais assertivo. O interesse em trazer os transportadores de membrana foi devido ao seu envolvimento com o metabolismo de drogas, mas também para avaliar a sua possível relevância no CC, já que não existem muitos dados sobre esses mecanismos na literatura científica.

2 CAPÍTULO I

Membrane transporter genes predict chemoradiotherapy response in cervical cancer patients

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Abstract

Introduction: Cervical cancer is women's fourth most common cancer worldwide. Improving survival remains the goal of the treatment in the clinical setting. Although some advances have been made in cervical cancer screening technologies, the survival rate and response to treatment remain poor. Membrane transporters encompass many proteins responsible for maintaining proper amounts of molecules inside and outside the cells. Among them, the family of ATPase and ATP synthase transporters stands out for their involvement in the process of resistance to chemotherapy and platinum accumulation in whole cells. Solute carrier proteins (*SLC*) have also been highlighted for playing critical roles in the movement of cytotoxic agents across the neoplastic cell membrane. The potential to determine efficacy, toxicity, and resistance to chemotherapy makes these transporters promising predictive biomarkers for cervical cancer. **Objective:** The present study aims to identify the expression profile of genes related to membrane transport as biomarkers to chemoradiotherapy in CC. **Methods:** Fluorescence-activated cell sorting (FACS) was used to isolate non-stem cells (NCCSCs) from cervical cancer biopsies. The cDNA library of samples from 21 responders (R) and 10 non-responders (NR) patients were constructed and sequenced using the Illumina platform. Expression analysis was performed using the DESeq2 package in the R program, considering differentially expressed genes (DEGs) for those with log fold change > 1 or < -1 and padj ≤ 0.05. Wald's statistical test and *p*-value adjustment with Benjamini's Hochberg method were also performed with the same tool. Decision trees with differentially expressed genes (DEGs) related to membrane transporters were analyzed using WEKA software. **Results:** Two large groups of genes with opposite differential expression profiles were revealed. The first group, composed mainly of the *SLC35, ATP1, and ATP5* families, showed overexpressed genes in NR patients compared to R patients. The second group, mainly represented by the *SLC25* and *ATP6* families, was overexpressed in R patients compared to NR patients. Decision tree methods define the profile of *ATP1B3* and *SLCOB3* genes as capable of classifying patients into responders and non-responders and cut-off of counts is necessary to this with an accuracy of 90% in LOOCV. The results suggest *ATP1B3* and *SLCOB3* signature-based are candidate predictors of response to chemoradiotherapy.

Keywords: cervical cancer, *SLC*, *ATPase*, chemoresistance

1 Introduction

Cervical cancer (CC) is the fourth most common cancer in women, with an estimated incidence of 6.5% of all cancers and a death rate accounting for almost 8% of all cancer deaths in women worldwide (1). Squamous cell carcinoma and adenocarcinoma account for about 70% and 25% of cervical cancers, respectively, with their prevalence varying across the world (2). The incidence and mortality rates of CC are significantly higher in low- and middle-income countries, with more than 85% of new cases and 80% of cervical cancer deaths occurring in these countries due to less effective screening programs and infrastructure for follow-up and treatment (3).

Among the various risk factors for this type of cancer, most are linked to exposure to human papillomavirus (HPV) (4). Other risk factors for CC may involve reproductive, sexual, and behavioral factors such as high parity, multiple sexual partners, smoking, and low socioeconomic status (5).

Survival rates depend on several factors, and the main one is the stage of CC when it is diagnosed. With early detection and treatment, the 5-year survival rate is 92%. At the stage where CC has reached adjacent tissues or organs and/or regional lymph nodes, the 5-year survival rate is 58%. Finally, in cases of distant metastases, the 5-year survival rate is only 17% (6).

Improved survival remains the ultimate treatment goal in the clinical setting. Although some advances have been made in CC screening technology and HPV vaccine delivery, the survival rate and response to treatment remain poor (7). Chemotherapy, surgery, radiation therapy, and brachytherapy are the standard treatment options for CC (8). However, even with the effectiveness of the treatment, some patients may resist it, making it difficult to choose the therapeutic follow-up. The mechanisms of the molecular changes involved in chemoresistance in CC are still not fully understood (9).

Membrane transport proteins appear to be potential predictive-prognostic tools in cancer, as events related to this transport are critical in determining cell fate for survival, death, or malignant transformation (10). They also balance toxicity and maintenance of chemotherapeutics, controlling their absorption and distribution between tissues. Therefore, alterations in the expression profile of membrane transport proteins may be associated with tumorigenesis and chemoresistance (11).

The Solute Carrier (*SLC*) genes superfamily is the second-largest family of membrane transporter proteins that play an essential role in maintaining homeostasis, absorbing solutes that do not freely diffuse across biological membranes, and transporting drugs (12). SLC genes are expressed in many types of cancer and can be differentially expressed between malignant and non-malignant tissues (13). Different expression patterns of these transporters have been associated with metastatic cancer, indicating their potential as therapeutic targets and for prognosis of drug-resistant metastatic disease. (14).

Adenosine triphosphatases (ATPases) and Adenosine triphosphate synthases (ATP synthase) are related to energy metabolism, and changes in their function are related to tumor angiogenesis, metastasis, and drug resistance (15). ATP-binding cassette (*ABC*) transporters, solute transporters, and ATPase membrane protein superfamilies have been described as direct influencers of platinum drug pharmacology (16) and may, in cells resistant, affect their accumulation by increasing efflux or decreased drug absorption (17, 18).

The present study aims to identify possible membrane transporter genes that correlate their differential expression pattern and the manifestation of resistance to chemoradiation therapy in patients with CC leading to more accurate decision-making and more efficient therapy.

2 Methods

2.1 Patient recruitment and sample selection

Patients of the Hospital Luxemburgo - Instituto Mário Penna with a diagnosis of CC of the squamous cell carcinoma or adenocarcinoma type without previous history of immunological diseases or CC diagnosis were recruited. A total of thirty-one patients were selected to meet these criteria. The tumor staging was performed according to the International Federation of Gynecology and Obstetrics (FIGO) recommendations. Samples were obtained from cervical biopsies collected before starting chemoradiotherapy treatment. The patients had their health monitored for eight months through imaging and cytohistological exams. Those who had cervical lesions during this period were considered non-responders (NR) to treatment, while those with no cervical lesions detected were considered responders (R). Thus, of the 31 patients, 21 were considered responders (R) and 10 non-responders (NR). The study was performed in accordance with the Ethical Committee for Research in Human Beings guidelines of the Institution (Number 1.583.784)**.** An informed consent from all patients involved was also obtained. Additional information about the selection process for these samples has already been described previously by Zuccherato et al., 2021 (19).

2.2 Non-stem cervical cancer cell (NSCCC) sorting by flow cytometry*

Our group (Zuccherato et al., 2021) has previously provided detailed descriptions of how non-stem cells were sorted. To obtain the non-stem cells, samples were fragmented and frozen in a specialized cryoprotective solution. Subsequently, flow cytometry was employed to separate stem and non-stem cells based on criteria such as fluorescent signals emitted, as well as the appearance and size of granules.

2.3 cDNA synthesis and preparation of Sequencing Libraries

RNA was extracted from non-stem cells and converted into cDNA using the SMART-Seq v4 Ultra Low Input RNA Sequencing Kit (Takara Bio USA, CA). Successful reverse transcription was confirmed and cDNAs with the best quality parameters were selected for library preparation. Sequencing was performed on the NextSeq® 550 sequencer (Illumina, CA) using a NextSeq® 500/550 High Output Kit v2. Details of these procedures can be found in Zuccherato et al., 2021.

2.4 Transcriptome analysis

The files obtained in RNA-Seq were firstly submitted to the quality control through the FastQC tool (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and the reads obtained passed through the trimming of adapters and filtering of sequences with poor quality (Phred score ≤ 35) through Cutadapt (20). A new analysis was then performed, also with the FastQC tool, on the sequences previously trimmed. Then, the read sequences were aligned with the human reference genome (Homo_sapiens.GRCh38.83) using the STAR software (21).

2.5 Analysis of differentially expressed genes (DEGs)

The analysis of differences in NSCCC mRNA expression between the Responder and Non_responder patients groups was performed using the "Bioconductor DESeq2 " package in the R program (22). The trimmed sequences were filtered and normalized using the HTSFilter (23) and the default normalization parameters of the DESeq2. After the analysis, the genes with adjusted P value ≤ 0.05 and logFoldChange > 1 or < -1 were considered differentially expressed.

2.6 Gene Ontology, DEGs selection, and heatmap visualization

The EnrichPlot (24) and Clusterprofiller R (25, 26) packages were used for Gene Ontology analysis. For the first analysis, all DEGs obtained from RNA-Seq were used. Based on this GO result, it was possible to proceed with selecting the most representative ontologies. DEGs related to the transport process were selected, and the relationships of these DEGs were obtained both with terms of biological process (BP) and with molecular function (MF). Only GO enriched terms with a padj value ≤ 0.05 were considered relevant. The selected data based on GO analysis were used to create heatmaps through the "pheatmap" R package (https://www.rdocumentation.org/packages/pheatmap/versions/1.0.12/topics/pheatmap). For that, the search terms “*SLC*”, “*ATP*”, “*ABC*” and “*OTC*” were used.

2.7 Machine Learning (ML) Algorithms and Overall Survival (OS)

Normalized counts of DEGs related to membrane transporters were employed to decision trees construction, using WEKA software (Waikato Environment for Knowledge Analysis, version 3.6.11, University of Waikato, New Zealand) (27). The classifier algorithms were trained to characterize patients as responders (R) and non-responders (NR) to treatment. Clinical characteristics such as outcome, metastasis, and FIGO classification were considered. To estimate the classification accuracy and test the model's generalization, leave-one-out cross-validation (LOOCV) was performed. Additional Kaplan-Meier plots with Log Rank (Mantel-

Cox) were performed to assess a possible association between the expression profile of the *ATP1B3* gene and Overall Survival (OS) using SPSS software (version 20, IBM, USA).

3 Results

3.1 Clinicopathological Characteristics of the Cohort

The patients in the study had squamous cell carcinoma-type CC as their primary diagnosis, with FIGO classification ranging from IIA, IIB, and IIIB. These are patients who have unilateral (29.03%) and bilateral (67.74%) parametrial involvement and vaginal involvement (87.10%) factors of poor prognosis.

Regarding treatment, all patients, except one, used cisplatin combined with brachytherapy as standard treatment. At four months of treatment, two patients presented metastasis. At eight months of treatment, the number of metastatic patients increased from 6,45% to 29,03% (Table 1). After eight months of treatment, 64,52% of patients responded to treatment, while 35,48% did not respond, presenting disease progression or recurrence.

Table 1 - Clinicopathological characteristics of the 31 CC patients in the study

Characteristics	N	%
DIAGNOSIS		
Adenocarcinoma	1	3,23
Squamous cell carcinoma	30	96,77
HISTOLOGICAL GRADE		
II	19	61,29
III	12	38,71
FIGO CLASSIFICATION		
IIA	1	3,23
IIB	13	41,93
IIIB	17	54,84
PARAMETRIAL INVOLVEMENT		
Unilateral	9	29,03
Bilateral	21	67,74
Free	1	3,23
VAGINAL INVOLVEMENT		
Present	27	87,10
Absent	2	6,45
Not applicable	2	6,45
ANTICANCER AGENT		
Cisplatin	30	96,77
Carboplatin	1	3,23
STATUS AFTER 8 MONTHS OF TREATMENT		
Responder	20	64,52
Non-Responder	11	35,48
METASTASIS		
After 4 months of treatment		
Present	2	6,45
Absent	29	93,55
After 8 months of treatment		
Present	9	29,03
Absent	18	58,07
Not applicable	4	12,90

TABLE I Description of the clinical characteristics of the 31 cervical cancer patients, including type of diagnosis, histological grade, FIGO classification, presence of parametrial or vaginal involvement, type of drug used in the treatment, outcome after 8 months of treatment and presence of metastasis.

3.2 Differentially expressed genes and gene ontology analysis

A total of 2,519 differentially expressed genes were found in the comparison between the NSCCC samples of R and NR patients, considering the parameter log2FoldChange >1 or < -1 and padj value ≤ 0.05. Among them, 41 genes were annotated as membrane transporters at the level of molecular function (MF) gene ontology (GO).

The Gene Ontology enrichment of the DEGs obtained in the sequencing revealed 390 terms related to biological processes (BP) (Figure 1) (Supplementary Table I). It is possible to perceive four main groups, and the one that stands out the most is that related to transport in the cell membrane. Therefore, this group was selected as the focus of this study.

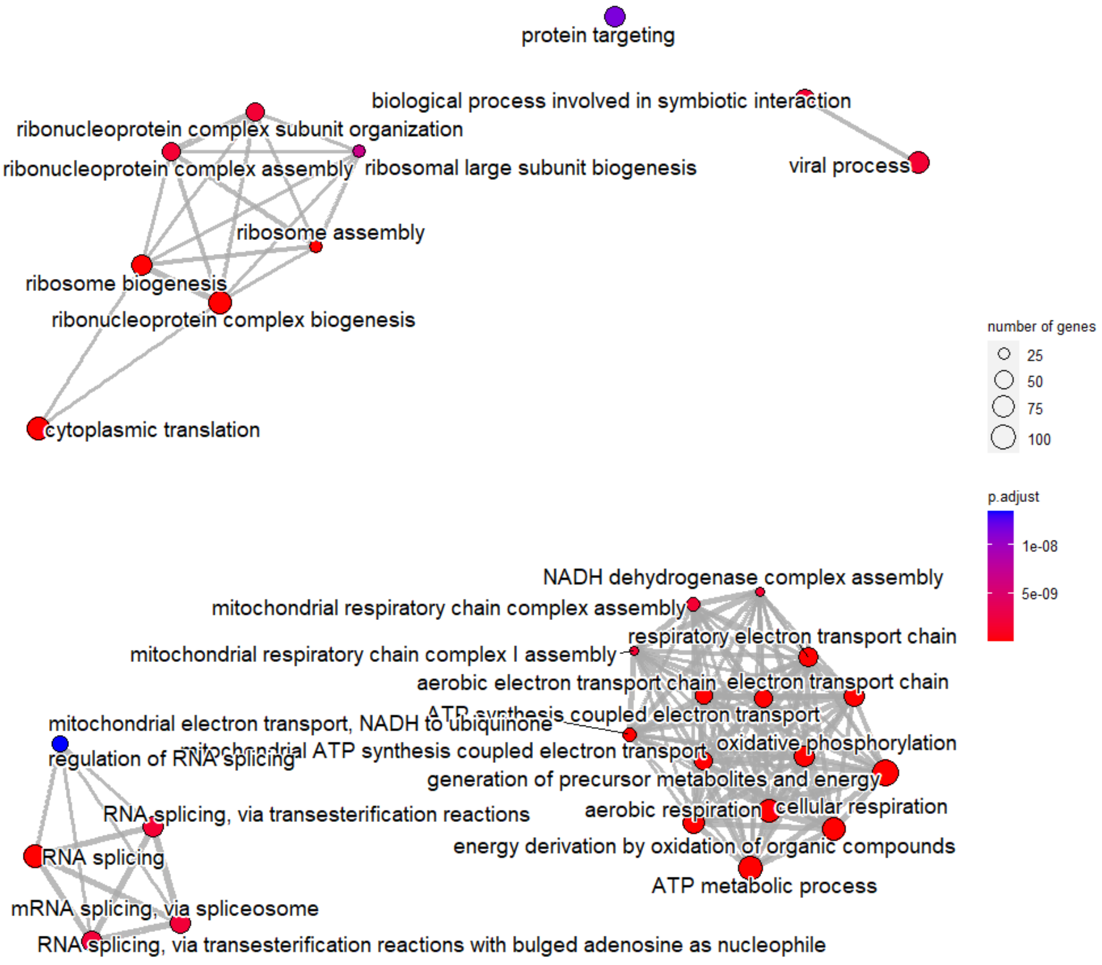

Figure 1 - Visualization of biological gene ontology terms for all genes with differential expression found in sequencing. The size of the nodes corresponds to the number of genes associated with a term. The significance (p adjusted value) is represented by the color of the nodes.

Performing a new GO analysis with these selected DEGS, most of the terms in BP are related to cellular energy metabolism, highlighting essential functions such as oxidative phosphorylation, ATP synthesis, and nucleotide metabolic processes (Figure 2A). About molecular function (MF), 50 terms were found. As expected, all of them are related to the transport of different substrates, with the main activity in the proton transmembrane transporter, ATPase-coupled transmembrane transporter activity, and active transmembrane transporter activity primary (Figure 2B).

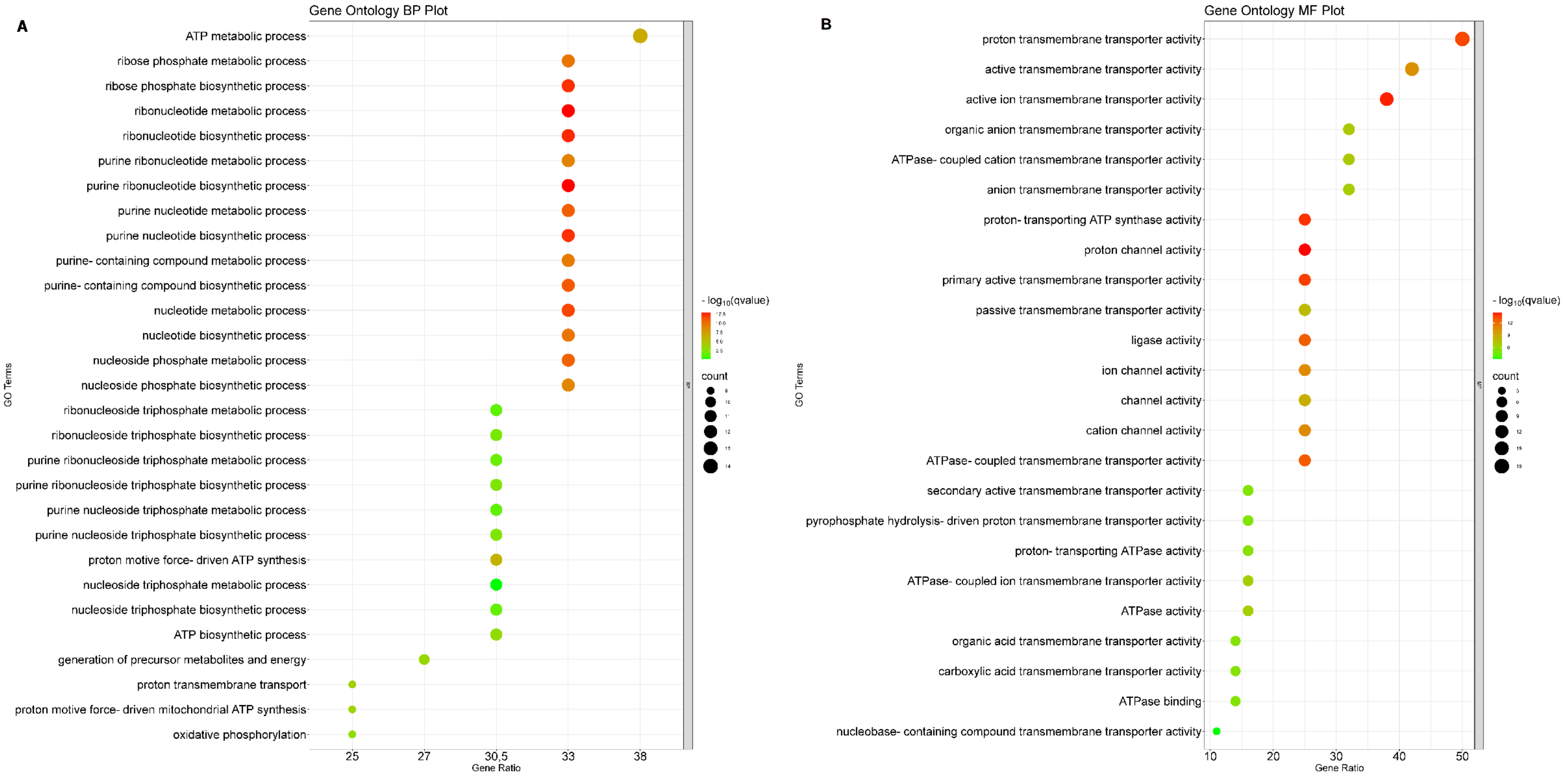

Figure 2 - Graphical representation of the main GOs classified according to gene count. The parameters used in the creation were the '-log10 (qvalue)', 'Gene count', the number of enriched genes in a GO term, and the 'Gene ratio' which is the percentage of the selected DEGs in the given GO term. **A** - Represents terms related to biological processes. **B** - Represents terms related to molecular function. GO, genetic ontology; BP, biological process; MF, molecular function.

Among the filtered DEGs (Supplementary Table I), four primary membrane transporters were identified, including SLC, ABC, P-type ATPases, and ATP synthases. Thirteen SLC genes, namely S*LC25A5*, *SLC25A39*, *SLC25A1*, *SLC9A3R1*, *SLC1A2*, *SLCO1B3*, *SLC2A4RG, SLC38A2*, *SLC49A4*, *SLC35G3*, *SLC25A6*, *SLC35G5*, and *SLC5A4P1*, showed differential gene expression, as did 27 genes belonging to ATP metabolism families, ATPases and ATP synthases. Additionally, the only upregulated gene in NR patients was *ABCA10*, belonging to the ATP-binding cassette (ABC) family.

The heatmap representative of the transporters' expression levels (Fig.3) uses the presence or absence of metastasis, FIGO staging, and the outcome of the patients (R or NR) as factors for gene clustering and demonstrated two large groups of genes with antagonistic differential expression profiles. The first group, composed mainly of the *SLC35, ATP1*, and *ATP5* families, presented genes overexpressed in NR compared to R. The second group, represented mainly by the *SLC25* and *ATP6* families, was overexpressed in R concerning NR. Additionally, the analysis is applicable to downregulated genes as well. These genes displayed opposing expression patterns in R and NR patients, with the same gene being under-expressed in responders and overexpressed in non-responders, and vice versa.

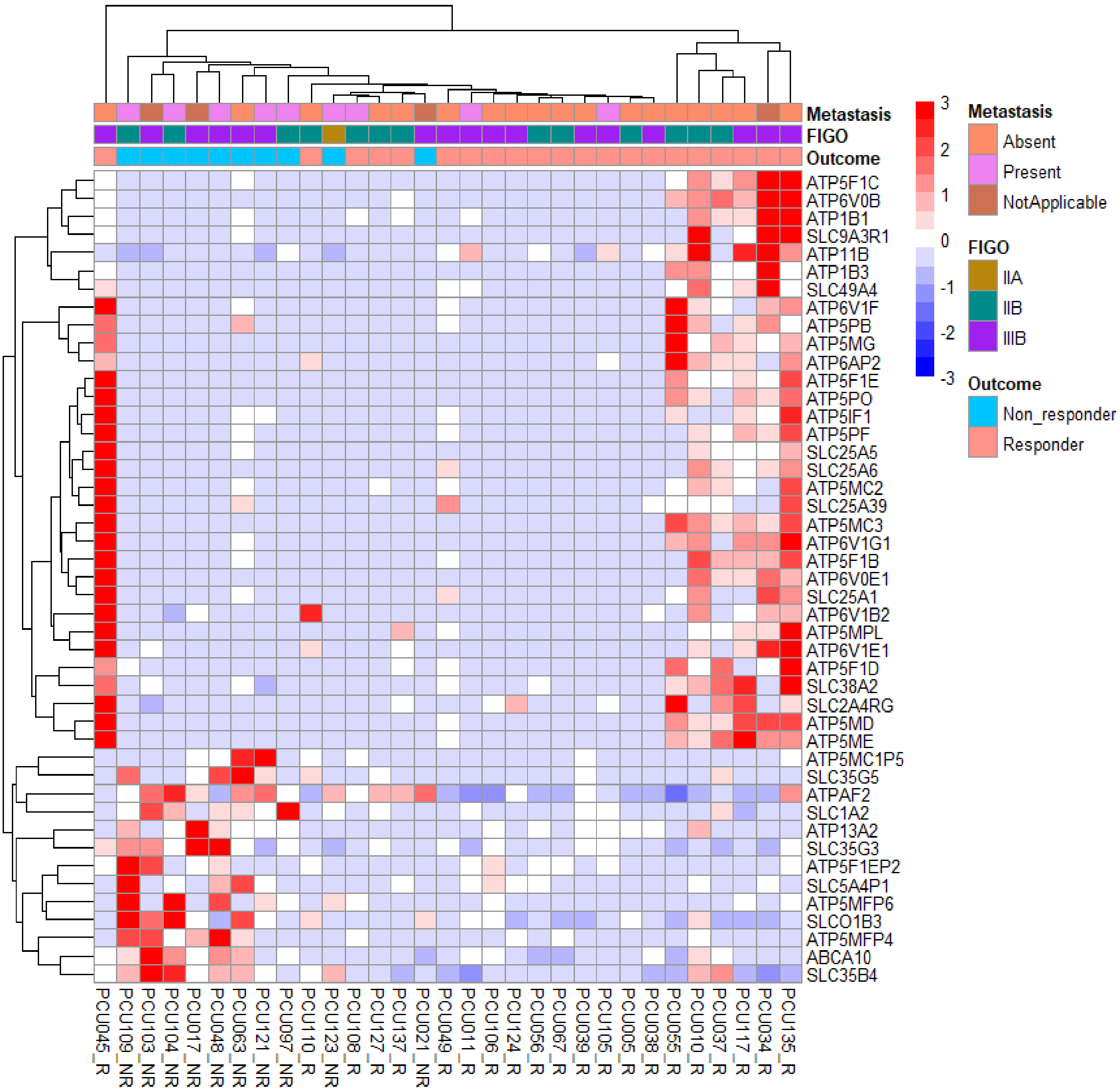

Figure 3 – Heatmap showing the membrane transporter selected differentially expressed genes (adjusted p-value ≤ 0.05) in patients with cervical cancer. Responders (R; n=21) are represented in salmon pink and non-responders (NR; n=10) are represented in blue. Other features such as presence of metastasis and FIGO classification are also detailed in color in the map legend.

3.5 Machine Learning and Overall Survival (OS)

The decision tree (Figure 4) demonstrated that the *ATP1B3* and *SLCO1B3* genes are efficient to classify patients as N and NR to treatment and determined the cut-off of gene expression level is implicated in this patients segregation. The results showed that the algorithm, employing only the *ATP1B3* gene, could identify 7 out of 10 non-responders (70%). The other patients were classified through the differential expression of the *SLCO1B3* gene, which, in turn, correctly classified the remaining patients into R and NR. The algorithm reached 31/31 possibilities (100%) using the complete data set (Full Training), and when this was evaluated with the LOOCV method, the algorithm was able to correctly classify 28/31 hits, having an accuracy of 90,32%.

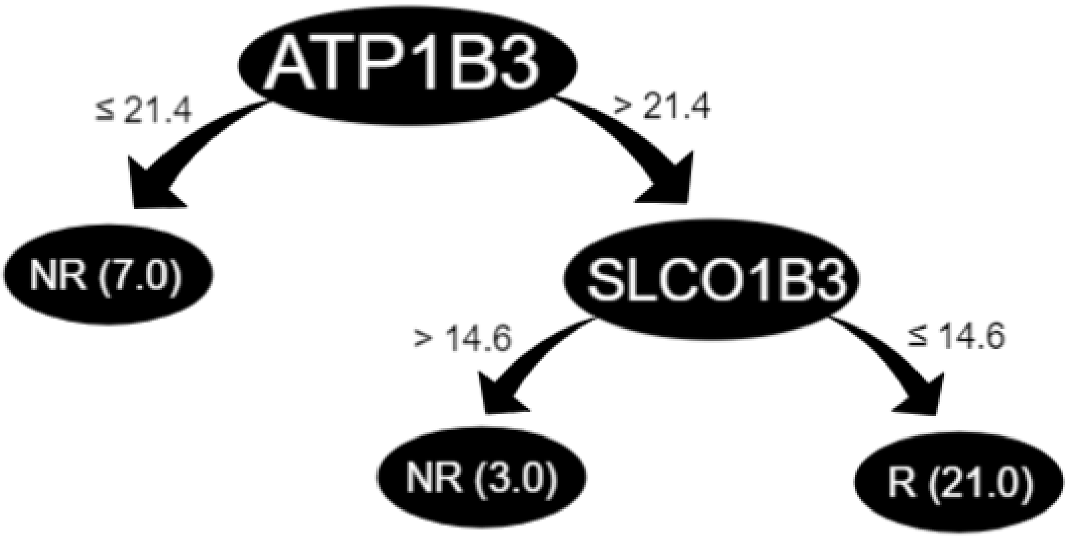

Figure 4 - Analysis of the decision tree via machine reading demonstrating that only the analysis of the expression of the *ATP1B3* and *SLCO1B3* genes are necessary for the segregation of patients into responders or non-responders. The *ATP1B3* gene alone was able to segregate 7 of the 10 non-responders with counts equal to or less than 21.4, while the *SLCO1B3* gene segregated the rest of the patients, using counts equal to or less than 14.6 as a criterion. R, responder; NR, non-responder.

The correlation of gene expression profile and t overall survival was represented in the Kaplan-Meier (OS) plots. Figure 5 shows that patients with the *ATP1B3* gene upregulated had a higher survival rate.(p-value 0.06).

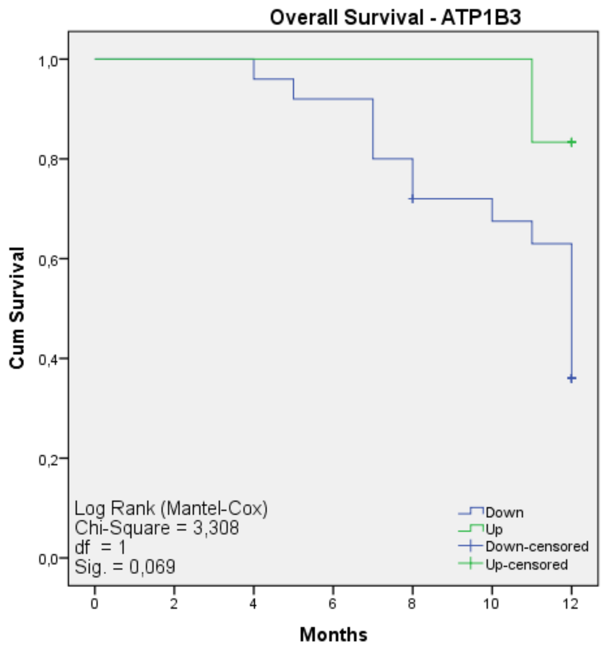

Figure 5 - Kaplan-Meier plot of overall survival for patients with cervical cancer identified by gene expression profiling of *ATP1B3*.

4 Discussion

The absence of biomarkers that can guide the medical team in tailoring the most appropriate treatment for each patient based on the intrinsic characteristics of their tumor poses a significant challenge. Chemotherapy resistance has been considered one of the main challenges in the fight against CC. Tumor cells are resistant to antineoplastic agents by molecular mechanisms, such as changes in drug accumulation within cells, increased drug efflux activity, and promotion of DNA repair capacity, which have been studied to improve the prognosis of patients with cancer (28).

This study intends to determine the potential of DEGs with functions related to membrane transport as biomarkers able to support clinical decision through the accurate prediction of the response to treatment. For this, we exploited DEG data from an NGS approach associated with clinical data using statistical and other ML methods.

The landscape of DEGs between the NR and R revealed 2,519 genes and GO results demonstrated an important enrichment of terms in biological processes and molecular function regarding the membrane transport activity for 41 of them. Most of the genes investigated in this study are crucial for cellular energy metabolism. Among them, the most prominent families are SLC, ATP synthase, ATPases, and ABC.

The SLC genes, a family of over four hundred membrane-bound transporters, have been widely targeted for drug development (29). ATPases are enzymes that hydrolyze ATP into ADP and a free phosphate ion (30), while ATP synthases are mitochondrial enzymes located in the inner membrane responsible for synthesizing ATP through electron transfer gradients (31). ABC transporters, which bind to ATP, play a role in various cellular processes, including cancer resistance, antigen processing, cell division, and immunity (32).

So far, the results show two groups of genes with antagonistic expression patterns between R and NR patients. The *ATP1*, *ATP5*, and *SLC25* family genes highlight those that stand out in responding patients. *ATP1* belongs to the subfamily of Na^+/K^+ ATPases (*NKA*), and its primary function is the translocation of sodium and potassium ions across the cell membrane. The alteration in the expression of isoforms of these enzymes is involved in developing human cancers (33).

The downregulation of *ATP1B1* gene expression, which encodes the β1 subunit of NKA, has been identified as a crucial factor in increasing the invasiveness of epithelial cancer cells, and is being studied as a potential tumor suppressor (34). On the other hand, *ATP1B3* gene, which encodes the β3 subunit of the enzyme, regulates cell adhesion and is implicated in promoting tumorigenicity through activation of pro-inflammatory factors in gastric cancer (35).

Our findings are consistent with previous research indicating that ATP1B1 may function as a potential suppressor of cancer progression, as evidenced by its upregulation in treatment-responsive patients without metastasis. However, the exact role of ATP1B3 in tumor cell resistance to antineoplastic drugs requires further investigation. In colon cancer, overexpression of functional ATP1B3 has been shown to decrease TPp53 transcriptional activity, resulting in apoptotic resistance in neoplastic cells (36). Additionally, the expression of ATP1B3 varies among different types of cancer, with upregulation observed in hepatocellular adenomas and focal nodular hyperplasia, and downregulation in liver metastases of pancreatic cancer (37). Although there is evidence of a positive association between ATP1B3 and tumorigenesis, our study suggests that this gene is critical for identifying patients who will respond well to treatment.

The SLC25 family supports oxidative phosphorylation and enables transport across the inner mitochondrial membrane, linking cytosolic and mitochondrial metabolism. Additionally, some members of this family may act as potential biomarkers for cancer and contribute to adaptive mechanisms that promote drug resistance in tumor cells (38).

SLC25A6 has been shown to induce apoptosis in human HeLa and HepG2 cells in various studies (39), while SLC25A5 has exhibited paradoxical effects on drug resistance in different types of cancer. It enhances resistance to tyrosine kinase inhibitors in lung cancer and reverses resistance to immunotherapy in hepatocellular carcinoma. Furthermore, upregulation of SLC25A5 has been associated with better overall survival in patients with colon cancer (40). Our findings support the evidence that up-regulated SLC25 family members may play an important role in drug resistance once it is observed in R patients only.

Tumor cells are known for their altered energy metabolism, with drug-resistant liver cells exhibiting increased mitochondrial energy metabolism and glycolysis. ATP5, a mitochondrial ATP synthase, plays a crucial role in catalyzing ATP synthesis via an electrochemical proton gradient during oxidative phosphorylation. Alterations in its expression can impact ATP production in treatment-resistant cells (41). In this study, overexpression of ATP5 family members was associated with a positive treatment response and a favorable prognosis in patients undergoing chemoradiotherapy.

ATP6 family members, which are part of the genes encoding V-ATPase components, play a role in facilitating intracellular acidification and proton extrusion (42). Changes in pH gradient between intracellular and extracellular compartments affect drug resistance mechanisms, including the lysosomal sequestration of chemotherapy drugs (43). Although additional investigations are required, this study showed the overexpression of *ATP6* family members, like in the previous analysis, could be considered as a predictive biomarker, indicating treatment responder patients.

The other group represents those genes that showed upregulation in non-responders patients, highlighted by the families *SLC35*, *SLCO*, and *ABC*. While *ABC* and *SLCO* transporters are generally related to the function of drug absorption, distribution, metabolism, and excretion, *SLC35* has been described mainly as involved in the absorption of sugars, impacting essential processes such as cell survival and homeostasis (44, 45).

High-level expression of *ABCA* genes has been associated with drug resistance and poorer patient outcomes in various cancers, such as neuroblastoma, acute lymphoblastic leukemia, and cancers of the prostate, lung, pancreas, and kidney. Furthermore, others indicate a relationship between the *ABCA10* gene and tumor immune infiltration, making it a potential prognostic marker (46, 47). In this study, the downregulation in R and upregulation of this gene in NR indicate a possible negative relationship between its gene expression level and the response to chemotherapy treatment. These results highlight the need for ABCA10 gene expression to be explored as a predictive tool.

The *SLCO* families are already known as targets of immunotherapies, due to their role in altering several drugs' pharmacokinetics through a series of pharmacogenomic polymorphisms (48). In our study, the *SLCO1B3* gene expression appears to be a potential predictive factor due to its overexpression in NR patients only, indicating a probable positive relationship with resistance to the cytotoxic agent used, cisplatin. However, further studies are needed to better understand its role in CC.

SLC35 family genes were overexpressed in NR, including *SLC35G5*, which has been shown to be upregulated in cisplatin-resistant lung cancer cells compared to cisplatin-sensitive cells (49). *SLC35G3* up-regulation in colorectal cancer tissues has been linked to poor patient prognosis, besides *SLC35B4* gene up-regulation was observed in multi-resistant hepatocellular carcinoma (HCC) cells, compared to non-resistant cells (50) These data support our study's findings that positive regulation of *SLC35* family genes may indicate a greater chemoresistance.

To confirm the great potential of transporters as predictive biomarkers for CC patients, decision trees from the selected DEGs were constructed and analyzed. Besides, the Kaplan Meier analyses were used to demonstrate the predictive value of the genes identified by ML methods. Upon close inspection, the best bioinformatic results point to the *ATP1B3* and *SLCO1B3* genes achieving high performance when applied to the ML classifiers algorithm. In the follow-up of the patients, besides the relationship between *ATP1B3* expression and overall survival is noticeable, no statistical differences were observed. There are studies of *ATP1B3* on its possible relationship with tumorigenesis, but there is still a lack of investigations on its relationship with chemoresistance.

5 Conclusion

The results presented on the expression of genes that encode membrane transporters are encouraging regarding their potential applicability as predictive biomarkers for CC. A comprehensive analysis of the expression patterns of these genes in the CC has not yet been carried out. A study focused on membrane transporters may clarify their role in biochemical functions and other mechanisms, such as tumor cell proliferation, apoptosis, and cellular drug influx. This could identify membrane transporters as potential predictive tools for CC or as molecular targets for developing anticancer drugs. The main limitations of our study were sample size and follow-up time, which could justify the p-value in the Kaplan Meier is not below 0.05, despite being close. Even so, it is possible to point out a possible gene signature of ATP1B3, with predictive value, for this type of cancer.

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3 CONCLUSÃO

Os resultados sobre os transportadores de membrana desses estudos são animadores quando falamos de potenciais biomarcadores prognósticos-preditivos. A combinação de análise de dados de Transcritoma, dados clínicos e dados de aprendizado de máquina apontam para uma possível assinatura genética para esse tipo de câncer. A possibilidade de identificar possível resistência ao tratamento padrão com cisplatina para pacientes com câncer de colo do útero seria de grande importância para subsidiar a decisão médica e melhorar a sobrevida dessas pacientes, tornando o tratamento mais eficaz.

4 PERSPECTIVAS

Para confirmar os achados encontrados nesse primeiro estudo, serão feitas novas análises em um maior número de amostras de câncer de colo de útero. O objetivo é realizar PCR em tempo real para confirmar o perfil de expressão dos genes ATP1B3 e SLCO1B3. A partir dos resultados obtidos nesse experimento novos objetivos serão traçados.

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APÊNDICE

Gene	Gene name	Biological Function	Mechanism in cancer progression/resistance	References
SLC25A5	Solute Carrier Family 25 Member 5	This gene encodes a mitochondrial protein that plays a crucial role in the regulation of the balance of adenine nucleotides in cells.	Studies have shown that SLC25A5 overexpression leads to the activation of survival signaling pathways and increased oxidative stress resistance in prostate cancer cells, resulting in enhanced resistance to chemotherapy.	https://doi.org/10.7150/thno.42874.
SLC25A39	Solute Carrier Family 25 Member 39	This gene encodes a mitochondrial protein that is involved in the transport of urea into the mitochondria	The SLC25A39 gene may play a role in cancer cell proliferation, but more research is needed to fully understand its potential impact on cancer progression and chemoresistance. It can be overexpressed in several types of cancer cells, including liver, stomach, and lung cancer cells, and its knockdown inhibited the growth of these cancer cells.	https://doi.org/10.1101/2021.09.15.460381
SLC25A1	Solute Carrier Family 25 Member 1	This gene encodes a mitochondrial protein that is involved in the transport of citrate from the cytoplasm into the mitochondria.	Alterations in SLC25A1 expression have been reported to contribute to cancer progression and chemoresistance due to increased energy metabolism and antioxidant defense in breast cancer cells, leading to increased resistance to chemotherapy.	https://doi.org/10.1038/s41388-019-0925-0.
SLC9A3R1	Solute Carrier family 9, subfamily A (NHE3,	This gene encodes a protein that regulates the function of the SLC9A3 gene, which encodes the NHE3 (Na+/H+ exchanger	Studies have shown that changes in SLC9A3R1 expression may contribute to cancer progression and chemoresistance and may be a predictor of poor prognosis and cause increased resistance to chemotherapy in ovarian cancer.	https://doi.org/10.3892/ol.2015.3796

Supplementary Table I Description of the name, biological function and role in the mechanism of cancer progression and resistance of selected membrane transporter genes with differential gene expression. a: Information provided by GeneCards – the human gene database.

	cation proton antiporter 3), Member 3 Regulator 1	3) protein. The NHE3 protein is involved in the regulation of intracellular pH and the balance of cations in cells.		
SLC1A2	Solute Carrier Family 1 Member 2	This gene encodes a protein that is involved in the transport of glutamate, an amino acid, into cells.	Evidence suggests an important role for SLC1A2 in the treatment of breast cancer, with resistant cancer cells promoting autophagy and import of aspartate and glutamate through this transporter.	https://doi.org/10.1016/j.celrep.2019.06.010
SLCO1B3	Solute Carrier Organic Anion Transporter Family Member 1B3	This gene encodes a protein that is involved in the transport of organic anions, such as drugs and toxins, across cell membranes.	The upregulation of SLCO1B3 has been observed in several types of cancer cells, including hepatocellular carcinoma (HCC) and ovarian cancer cells. The overexpression of SLCO1B3 has been linked to increased resistance to chemotherapy and poor prognosis in cancer patients.	https://doi.org/10.1186/s12957-020-01891-y
SLC2A4RG	Solute Carrier Family 2 Member 4 Regulatory	This gene encodes a regulatory subunit of a protein that is involved in the transport of glucose across cell membranes.	There is limited information available on the role of SLC2A4RG in cancer progression and chemoresistance. However, the role of the protein encoded by this gene in glucose metabolism and cellular energy production has been extensively studied. Glucose is a major energy source for cancer cells, and alterations in glucose metabolism have been linked to cancer progression.	https://doi.org/10.1016/j.bbcan.2012.12.004
SLC38A2	Solute Carrier	This gene encodes a protein involved in the	Upregulation of SLC38A2 has been associated with increased chemotherapy resistance and poor prognosis in cancer patients. Overexpression of this gene can lead	https://doi.org/10.1002/asia.202001056

Supplementary Table I Description of the name, biological function and role in the mechanism of cancer progression and resistance of selected membrane transporter genes with differential gene expression. a: Information provided by GeneCards – the human gene database.

	Family 38 Member 2	transport of amino acids across cell membranes.	to the transport of amino acids, including some drug targets, into cancer cells, reducing the effectiveness of chemotherapy drugs.	
SLC49A4	Solute Carrier Family 49 Member 4	This gene encodes a protein involved in the transport of nucleosides across cell membranes.	Little evidence relates the SLC49A4 gene to cancer, but some studies have already demonstrated its relationship with some important processes, such as promoting oxidative stress and lipid peroxidation, leading to membrane damage and, ultimately, to apoptosis.	https://doi.org/10.3389/fphar.2014.00061
SLC35G3	Solute Carrier Family 35 Member G3	This gene encodes a protein involved in the transport of nucleotide sugars across cell membranes.	-	-
SLC25A6	Solute Carrier Family 25 Member 6	This gene encodes a protein involved in the transport of adenine nucleotides across mitochondrial membranes	Several studies have suggested that alterations in the expression of SLC25A6 is associated with resistance to chemotherapy in breast cancer cells by alterations in mitochondrial function and energy metabolism, affecting the cell's ability to respond to chemotherapy drugs.	https://doi.org/10.3389/fcell.2019.00003
SLC35G5	Solute Carrier Family 35 Member G5	This gene encodes a protein involved in the transport of nucleotide sugars across cell membranes.	-	
ATP11B	ATPase Phospholipid Transporting 11B	This gene encodes a protein that functions as a transmembrane transporter for a variety of substrates,	In cancer, ATP11B has been found to play a role in regulating tumor cell proliferation, survival, and metastasis, and has been implicated in chemoresistance in various types of cancer. A study found that ATP11B	https://doi.org/10.1172/jci149893

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		including lipids, ions, and drugs	expression was upregulated in ovarian cancer cells resistant to cisplatin, a commonly used chemotherapy drug.	
ATP1B3	ATPase Na+/K+ Transporting	This gene encodes a subunit of the Na+/K+ ATPase, which is an integral membrane protein that plays a crucial role in maintaining ion gradients across the plasma membrane of cells.	Studies suggest that ATP1B3 plays a role in cancer progression and chemoresistance. ATP1B3 expression was upregulated in ovarian cancer cells resistant to cisplatin, and that downregulation of ATP1B3 increased the sensitivity of the cells to the drug. Another study showed that ATP1B3 expression was higher in breast cancer cells that were resistant to the chemotherapeutic drug paclitaxel, and that downregulation of ATP1B3 sensitized the cells to the drug.	https://doi.org/10.3892/mmr.2019.10605 https://doi.org/10.3390/ijms231911102
ATP5F1B	ATP Synthase F1 Subunit Beta	This gene that encodes a subunit of the ATP synthase complex, which is involved in the generation of ATP.	ATP5F1B was found overexpressed and associated with poor survival in breast cancer and has been shown to predict longer survival in colon cancer.	https://doi.org/10.1186/1472-6890-13-30
ATP6V0E1	ATPase H+ Transporting V0 Subunit E1	This gene is a gene that encodes a subunit of the vacuolar ATPase (V-ATPase) enzyme, which is involved in proton transport across cellular membranes.	Overexpression of ATP6V0E1 has been linked to increased metastasis and resistance to chemotherapy in breast cancer. In ovarian cancer, ATP6V0E1 overexpression has been correlated with chemoresistance and poor patient prognosis. In pancreatic cancer, ATP6V0E1 has been identified as a potential therapeutic target.	https://doi.org/10.1158/0008-5472.can-04-3822 https://doi.org/10.1038/srep12465
ATP6V0B	ATPase H+ Transporting	This gene encodes a subunit of the vacuolar	ATP6V0B gene expression was up regulated in hepatocellular carcinoma (HCC) tumor samples and	https://doi.org/10.1155/2022/3594901

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	V0 Subunit B	ATPase (V-ATPase), which is a protein complex responsible for acidification of intracellular compartments, including lysosomes, Golgi apparatus, and endosomes.	positively correlated with risk scores and risk ratios, implying that increased oxidative phosphorylation activity may be associated with poor prognosis in certain subgroups of HCC.	
ATP5F1E	ATP Synthase F1 Subunit Epsilon	This gene encodes a is a nuclear-encoded subunit of the mitochondrial ATP synthase complex, which is involved in oxidative phosphorylation, the process by which cells produce ATP from the energy released by the electron transport chain.	Overexpression of ATP5F1E was associated with poor prognosis in lung adenocarcinoma patients and increased resistance to cisplatin, a commonly used chemotherapy drug. Studies also suggested that ATP5F1E may regulate the activity of the mitochondrial permeability transition pore, which is involved in apoptosis and cell death and may play a role in regulating the Warburg effect, a metabolic shift that allows cancer cells to survive in low-oxygen environments.	https://doi.org/10.3892/or.2019.7179 https://doi.org/10.1111/jcmm.14639
ATP6V1F	ATPase H+ Transporting V1 Subunit F	This gene encodes a subunit of vacuolar ATPase (V-ATPase), a multisubunit enzyme complex that regulates the pH of intracellular compartments by pumping protons across membranes.	Altered ATPase subunit expression and dysregulated ATPase activity have been linked to the occurrence, proliferation and invasion of various tumors and studies have shown that ATP6V1s family members are abnormally expressed in tumor tissues or tumor cell lines.	https://doi.org/10.3389/fonc.2020.567970

Supplementary Table I Description of the name, biological function and role in the mechanism of cancer progression and resistance of selected membrane transporter genes with differential gene expression. a: Information provided by GeneCards – the human gene database.

ATP5IF1	ATP Synthase Inhibitory Factor Subunit 1	This gene encodes for ATP synthase inhibitory factor 1, which is a mitochondrial protein involved in the regulation of the ATP synthase enzyme.	ATP5IF1 has been shown to be involved in chemoresistance in cancer cells by promoting cancer cell survival in response to chemotherapy, inhibiting the production of reactive oxygen species (ROS) and maintaining mitochondrial membrane potentiality.	https://doi.org/10.1074/jbc.m110.146480 https://doi.org/10.1113/jphysiol.2003.049478
ATP6V1E1	ATPase H+ Transporting V1 Subunit E1	This gene encodes a subunit of the vacuolar ATPase (V-ATPase), which is an enzyme responsible for regulating the pH of cellular compartments.	High levels of V-ATPase V1E1 expression were associated with worse prognosis in esophageal squamous cell carcinoma (SCC) and V-ATPase V1E1 depletion resulted in reduced cell motility, decreased glucose uptake, decreased levels of lactate, and decreased ATP production, as well as inhibition of glycolytic enzyme expression in TE8 esophageal cancer cells.	https://doi.org/10.18632/oncotarget.10340
ATP5MC2	ATP Synthase Membrane Subunit C Locus 2	This gene encodes for ATP synthase membrane subunit c locus 2, which is a subunit of the ATP synthase enzyme complex that is essential for mitochondrial oxidative phosphorylation and ATP synthesis.	-	-
ATP6V1G1	ATPase H+ Transporting V1 Subunit G1	This gene encodes a subunit of the vacuolar H+ ATPase (V-ATPase) enzyme, which is involved	Studies have suggested that ATP6V1G1 high expression of has been associated with poor prognosis and chemotherapy resistance in various types of cancer, including ovarian cancer, breast cancer, and gastric	https://doi.org/10.2147/ott.s102422 https://doi.org/10.18632/oncotarget.11805

Supplementary Table I Description of the name, biological function and role in the mechanism of cancer progression and resistance of selected membrane transporter genes with differential gene expression. a: Information provided by GeneCards – the human gene database.

		in the regulation of intracellular pH, membrane transport, and cellular signaling.	cancer by its involvement in lysosomal acidification. Lysosomal acidification is required for the activation of certain chemotherapeutic drugs, and ATP6V1G1 has been shown to play a key role in maintaining lysosomal pH.	
ATP1B1	ATPase Na+/K+ Transporting Subunit Beta 1	This gene gene encodes for the beta-1 subunit of the Na+/K+-ATPase, which is an ion transporter that maintains ion gradients across the plasma membrane	Reduced expression of ATP1B1 is associated with oxaliplatin resistance in cancer cells and appears to play an important role in sensitization of cells to oxaliplatin.	https://doi.org/10.1007/s00280-009-0985-x
ATP6V1B2	ATPase H+ Transporting V1 Subunit B2	This gene encodes a subunit of the V-ATPase (vacuolar H+ ATPase) complex, which is a proton pump responsible for acidifying cellular compartments.	Mutations in ATP6V1B2 may increase the autophagy flux associated with human follicular lymphoma cells. These changes allow lymphoma cells to survive under nutrient-poor conditions and block the process of self-feeding and self-preservation with drugs that kill mutant lymphoma cells.	https://doi.org/10.1038/s41417-022-00477-y
ATP5MC3	ATP Synthase Membrane Subunit C Locus 3	This gene encodes for the subunit c of the mitochondrial ATP synthase complex, which is responsible for the generation of ATP in the electron transport chain.	ATP5MC3 was used as a prognostic model of ferroptosis-related genes in colorectal cancer (CC), which predict the probability of survival of patients with CC.	https://doi.org/10.3389/fonc.2021.654076

Supplementary Table I Description of the name, biological function and role in the mechanism of cancer progression and resistance of selected membrane transporter genes with differential gene expression. a: Information provided by GeneCards – the human gene database.

ATP5PF	ATP Synthase Peripheral Stalk Subunit F6	This gene encodes a subunit of ATP synthase, a protein complex that is responsible for producing ATP, the primary source of energy for cellular processes.	Overexpression of ATP5PF can promote colorectal cancer metastasis, potentially through transactivation of serine-threonine protein kinase AKT2.	https://doi.org/10.1016/j.bbadis.2020.165846
ATP5MPL	ATP Synthase Membrane Subunit J	This gene encodes a subunit of the mitochondrial ATP synthase complex, which is responsible for the generation of ATP in the electron transport chain.	-	-
ATP13A2	ATPase Cation Transporting 13A2	This gene encodes a transmembrane protein which is involved in the transport of cations such as zinc, calcium, and manganese.	ATP13A2 is related to biogenesis and exosome release. In turn, exosomes can be released from cancer cells and are involved in cancer progression.	https://doi.org/10.1523/jneurosci.1629-14.2014 https://doi.org/10.33160/yam.2019.06.002

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ATP5F1C	ATP Synthase F1 Subunit Gamma	This gene encodes a subunit of the mitochondrial ATP synthase complex, which is responsible for the generation of ATP in the electron transport chain.	ATP5F1C is closely associated with growth, proliferation, and metastasis. Furthermore, it may be a prognostic biomarker of tumor recurrence and distant metastasis, as well as a marker of therapeutic failure in estrogen receptor-positive patients undergoing tamoxifen therapy.	https://doi.org/10.1038/s41418-021-00788-x
ATP5MG	ATP Synthase Membrane Subunit G	This gene encodes for a subunit of the ATP synthase enzyme, which is responsible for the synthesis of ATP in the mitochondrial inner membrane.	ATP5MG may play a role in the regulation of apoptosis in lung cancer cells and studies found that knockdown of ATP5MG expression led to an increase in apoptosis in lung cancer cells, suggesting its potential as a therapeutic target for the treatment of lung cancer.	https://doi.org/10.3892/ol.2017.5622
ATP5ME	ATP Synthase Membrane Subunit E	This gene is a nuclear-encoded subunit of the F-type ATPase complex, which is an important enzyme that synthesizes ATP from ADP and inorganic phosphate in the mitochondrial inner membrane.	-	-
ATP5MD	ATP Synthase	This gene encodes a subunit of the	ATP5MD is highly expressed in several cancer types and has been identified to promote cancer progression.	https://doi.org/10.1097/md.0000000000021478

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	Membrane Subunit K	mitochondrial ATP synthase complex, which is responsible for the production of ATP.		
ATP6AP2	ATPase H+ Transporting Accessory Protein 2	This gene encodes for a protein called ATPase H+-transporting accessory protein 2, also known as the (pro)renin receptor. The protein has been implicated in a range of cellular processes, including intracellular trafficking and endocytosis, and is involved in the renin-angiotensin system.	Recent studies have suggested that ATP6AP2 gene is involved in cancer progression and chemoresistance, promoting tumor growth and metastasis by activating signaling pathways, such as the extracellular signal-regulated kinase (ERK) and phosphatidylinositol-3 kinase (PI3K)/AKT/mTOR pathways.	https://doi.org/10.2147/cmar.s270024

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