

UNIVERSIDADE FEDERAL DE UBERLÂNDIA – UFU
INSTITUTO DE BIOTECNOLOGIA
PÓS-GRADUAÇÃO EM BIOTECNOLOGIA

BRUNA CUSTÓDIO DIAS DUARTE

**PERFIL DE EXPRESSÃO DE LNCRNAs E SUA RELAÇÃO COM A RESPOSTA À
QUIMIOTERAPIA EM CÂNCER DE COLO UTERINO: POTENCIAIS
BIOMARCADORES PARA DIAGNÓSTICO E PROGNÓSTICO**

PATOS DE MINAS – MG
FEVEREIRO DE 2024

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Dissertação de Mestrado apresentada ao
Programa de Pós-graduação em Biotecnologia
como requisito parcial para obtenção do título de
Mestre em Biotecnologia.

Orientador: Prof. Dr. Matheus de Souza Gomes

Coorientador: Dr. Fábio Ribeiro Queiroz

PATOS DE MINAS – MG

FEVEREIRO DE 2024

Ficha Catalográfica Online do Sistema de Bibliotecas da UFU
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D812 2024	Duarte, Bruna Custódio Dias, 1998- Perfil de expressão de lncRNAs e sua relação com a resposta à quimiorradioterapia em câncer de colo uterino: potenciais biomarcadores para diagnóstico e prognóstico [recurso eletrônico] / Bruna Custódio Dias Duarte. - 2024. Orientador: Matheus de Souza Gomes. Coorientador: Fábio Ribeiro Queiroz. Dissertação (Mestrado) - Universidade Federal de Uberlândia, Pós-graduação em Biotecnologia. Modo de acesso: Internet. Disponível em: http://doi.org/10.14393/ufu.di.2024.122 Inclui bibliografia. Inclui ilustrações. 1. Biotecnologia. I. Gomes, Matheus de Souza, 1981-, (Orient.). II. Queiroz, Fábio Ribeiro, 1985-, (Coorient.). III. Universidade Federal de Uberlândia. Pós-graduação em Biotecnologia. IV. Título. CDU: 60
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Bibliotecários responsáveis pela estrutura de acordo com o AACR2:

Gizele Cristine Nunes do Couto - CRB6/2091
Nelson Marcos Ferreira - CRB6/3074

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Mestre em Biotecnologia.

Aprovado em __/__/__

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PATOS DE MINAS – MG

2024



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Programa de Pós-Graduação em:	Biotecnologia				
Defesa de:	Dissertação de Mestrado Acadêmico				
Data:	29/02/2024	Hora de início:	09:00	Hora de encerramento:	11:00
Matrícula do Discente:	42212BTC002				
Nome do Discente:	Bruna Custódio Dias Duarte				
Título do Trabalho:	LncRNAs associados à resposta terapêutica ao tratamento quimiorradioterápico no câncer de colo uterino				
Área de concentração:	Biociências				
Linha de pesquisa:	Bioinformática e Biologia Molecular aplicada à genômica, transcriptômica e proteômica				
Projeto de Pesquisa de vinculação:	Bioinformática e Biologia Molecular aplicada à genômica, transcriptômica e proteômica				

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Iniciando os trabalhos o(a) presidente da mesa, Dr. Matheus de Souza Gomes, apresentou a Comissão Examinadora e a candidata, agradeceu a presença do público, e concedeu ao(à) Discente a palavra para a exposição do seu trabalho. A duração da apresentação do(a) Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

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Referência: Processo nº 23117.015151/2024-36

SEI nº 5220088

Dedico esta dissertação com o objetivo de obtenção do título de Mestre em Biotecnologia, primeiramente, ao **Pai Celestial**, pois sei de onde veio o acalento de que tanto precisei. Por vezes, conversava e desabafava olhando para o céu, pedindo por força e sabedoria, para conseguir concluir meus objetivos mesmo em meio às adversidades.

Dedico também aos **meus pais, minha mãe, Maria Angélica**, sempre fez tudo o que podia para fornecer uma educação de qualidade e me ajudou das mais diversas maneiras nesses anos longe de casa; e **meu pai, Eline Flávio**, pela inspiração profissional, afinal, estudo na mesma universidade que meu pai se formou, e por me ajudar a me manter nesses anos de estudo.

Dedico à minha alma gêmea, **minha irmã, Amanda**, que sempre me apoiou, se preocupou, me defendeu e me protegeu. Ela é a minha metade, minha inspiração e um dos grandes motivos de eu seguir nessa jornada.

Dedico à **minha namorada, Marcela**, que há cinco anos está comigo, lutando as minhas lutas, sacrificando finais de semana para me acompanhar nos estudos, e que tem toda a paciência do mundo.

Dedico também, às minhas amigas, irmãs de caminhada, **Bruna Cássia e Gabriela Tolentino**. Nós nos apoiamos, ajudamos e cuidamos. Acompanhamos todos os momentos, dos tristes aos felizes, como a chegada da “bebê biotec”, a **Isadora**.

Por fim, dedico este trabalho, à todas as **mulheres que lutam contra o câncer de colo uterino**. Continuarei me esforçando para auxiliar, de alguma forma, na sua luta!

AGRADECIMENTOS

Agradeço a **Deus** pelo dom da vida, pelas pessoas com as quais fui abençoada e pela minha saúde.

Agradeço **aos meus pais, Maria Angélica e Eline Flávio**, pela oportunidade do estudo, pela educação, pela minha saúde, criação e por todos os sacrifícios para que eu pudesse focar nos estudos. Eu agradeço de todo o meu coração, sei que palavras não são o suficiente, mas prometo continuar tentando ser a melhor pessoa que eu puder e lhes mostrar por meio de minhas ações, a minha gratidão. É por vocês e para vocês! Que Deus me dê saúde e tempo para conseguir lhes retribuir por tanto.

Agradeço à **minha irmã, Amanda**, a minha pessoa, que me entende como ninguém e que já se sacrificou muito por mim. O nosso amor de irmã e a nossa conexão são raridades. Sou tão grata por ter você. Obrigada por tudo!

Meus agradecimentos se estendem à **minha namorada, Marcela**, que tanto me acolheu nas minhas crises de ansiedade (que foram muitas), teve paciência e sempre busca me entender.

Bruna Cássia e Gabriela Tolentino, agradeço a amizade construída em meio ao caos do mestrado. O apoio e a força que demos uma à outra foram pilares para me manter de pé nesse processo. E claro, agradeço a **Isadora**, por alegrar as nossas vidas.

Agradeço às minhas colegas de república **Kaisy, Celeste e Mikaelly**, por tanta ajuda no dia a dia e por terem paciência com meu caos e minha ansiedade.

Ao meu orientador, **Dr. Matheus de Souza Gomes**, agradeço por me apresentar ao mundo da bioinformática e, principalmente, pelas oportunidades. Poder trabalhar em parceria com o Instituto Mário Penna e conhecer grandes pesquisadores da pesquisa translacional e oncológica, com certeza, foi um marco na minha vida. Estou sob sua orientação desde a graduação, da qual eu saí com um artigo publicado como coautora. Agradeço por me aceitar durante todos esses anos, por confiar a mim trabalhos de grande relevância e por não duvidar da minha capacidade, quando eu mesma duvidava. Que possamos seguir com a nossa parceria por mais tempo!

Agradeço ao meu coorientador, **Dr. Fábio Ribeiro Queiroz**, que foi um presente mais que especial. Sou grata por encontrar alguém no meio acadêmico com quem conseguisse me conectar e me sentir acolhida. Com você aprendi que o “dar errado” também é um resultado, e que disso posso tirar informações muito relevantes. Sou grata, pois, sem saber, você me salvou inúmeras vezes, com suas palavras encorajadoras e o seu reconhecimento. Muito obrigada pelas oportunidades e espero poder contar com a sua parceria nas próximas etapas.

Agradeço aos professores **Dr. Laurence** e **Dr. Pedro** pela participação nesse projeto. Ao meu colega, **Me. Angelo Borges de Melo Neto**, minha sincera gratidão por ter se disponibilizado a me ensinar tanto.

Ao **Milton**, por sempre me socorrer quando meu computador apresentava problemas. E, claro, jamais me esquecerei do tempo que te fiz passar resolvendo a instalação do *software* de alinhamento, obrigada! Você resolve absolutamente tudo, é criativo e persistente, por isso me inspiro tanto em você.

Agradeço ao **LBAM** pela estrutura. E ao **PPGBIOTEC** e ao **Instituto Mário Penna** pela oportunidade de desenvolver uma pesquisa tão relevante.

Agradeço à **FAPEMIG** pelo fornecimento da bolsa, a qual me permitiu realizar o mestrado.

Agradeço a todas as pessoas que, de alguma forma, colaboraram para que este trabalho pudesse acontecer e que me apoiaram nessa jornada!

RESUMO

O câncer de colo do útero (CCU) é predominantemente induzido por infecções persistentes de certos genótipos oncogênicos do papilomavírus humano (HPV) representando um desafio significativo para saúde pública mundial. A Organização Mundial da Saúde (OMS) estabeleceu um plano estratégico visando a eliminação do CCU como um problema de saúde pública até o ano de 2100. Contudo, apesar da comprovada eficácia da vacina contra o HPV, a implementação em escala global enfrenta obstáculos devido à limitada acessibilidade à vacinação e à prevalência desinformação. O tratamento do CCU é influenciado pelo estágio da doença, com um prognóstico frequentemente desfavorável em casos de doença avançada ou recorrente. Sendo assim, a descoberta e a aplicação de biomarcadores no CCU são fundamentais na decisão clínica, permitindo uma abordagem mais direcionada, melhorando a eficácia terapêutica e otimizando o monitoramento do progresso da doença. O presente estudo buscou explorar o potencial dos *long non-coding* RNAs (lncRNAs) no CCU, concentrando identificação e caracterização desses lncRNAs, na elucidação de seus mecanismos de regulação e interações moleculares, e na análise de seus padrões de expressão em pacientes que apresentaram respostas variáveis à quimiorradioterapia. Para tanto, células não-tronco de CCU foram isoladas utilizando sorting por citometria de fluxo e utilizadas para extração de RNA total. O RNA foi utilizado para construção de bibliotecas que foram posteriormente sequenciadas utilizando o Illumina Nextseq 550. A análise diferencial de expressão revelou 417 lncRNAs com padrões de expressão distintos entre as amostras de pacientes que apresentaram resposta ao tratamento e aquelas que não responderam. As análises revelaram que esses lncRNAs interagem significativamente com várias biomoléculas que são cruciais para a progressão do CCU e resistência terapêutica. Análises estatísticas permitiram correlacionar o perfil de expressão desses lncRNAs com a eficácia do tratamento. Os lncRNAs *ENSG00000267838*, *ENSG00000266340* e *FRMD6-AS1*, demonstraram influenciar a resposta ao tratamento quimiorradioterápico por meio do seu perfil de expressão. O *ENSG00000267838*, conseguiu diferenciar os grupos de respondedoras e não respondedoras através da sua regulação negativa e positiva, respectivamente. Estes resultados indicam que tais lncRNAs podem exercer um papel fundamental na modulação da resposta terapêutica, com potencial para atuarem como biomarcadores no diagnóstico, prognóstico e monitoramento da progressão do carcinoma cervical. Contudo, é imperativo a realização de estudos adicionais e processos de validação para corroborar essas evidências.

Palavras-chave: long non-coding RNAs; câncer de colo uterino; expressão gênica; perfil regulatório; biomarcadores; bioinformática.

ABSTRACT

Cervical cancer (CC) is predominantly induced by persistent infections of certain oncogenic genotypes of the human papillomavirus (HPV) and represents a significant global public health challenge. The World Health Organization (WHO) has established a strategic plan aimed at eliminating CC as a public health problem by the year 2100. However, despite the proven efficacy of the HPV vaccine, implementation on a global scale faces obstacles due to limited accessibility to vaccination and prevalent misinformation. The treatment of CC is influenced by the stage of the disease, with an often unfavorable prognosis in cases of advanced or recurrent disease. Therefore, the discovery and application of biomarkers in CC is fundamental to clinical decision making, allowing for a more targeted approach, improving therapeutic efficacy and optimizing the monitoring of disease progression. The present study sought to explore the potential of long non-coding RNAs (lncRNAs) in CC, focusing on the identification and characterization of these lncRNAs, the elucidation of their regulatory mechanisms and molecular interactions, and the analysis of their expression patterns in patients who showed variable responses to chemoradiotherapy. To this end, non-stem cells from CCU were isolated using flow cytometry sorting and used for total RNA extraction. The RNA was used to construct libraries which were subsequently sequenced using the Illumina Nextseq 550. Differential expression analysis revealed 417 lncRNAs with distinct expression patterns between samples from patients who responded to treatment and those who did not. The analyses revealed that these lncRNAs interact significantly with several biomolecules that are crucial for the progression of CC and therapeutic resistance. Statistical analyses made it possible to correlate the expression profile of these lncRNAs with treatment efficacy. The lncRNAs ENSG00000267838, ENSG00000266340 and FRMD6-AS1 were shown to influence the response to chemoradiotherapy treatment through their expression profile. ENSG00000267838 was able to differentiate the groups of responders and non-responders through its negative and positive regulation, respectively. These results indicate that these lncRNAs may play a fundamental role in modulating the therapeutic response, with the potential to act as biomarkers in the diagnosis, prognosis and monitoring of the progression of cervical carcinoma. However, additional studies and validation processes are imperative to corroborate this evidence.

Keywords: long non-coding RNAs; cervical cancer; gene expression; regulatory profile; biomarkers; bioinformatics.

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CAPÍTULO 1

1 INTRODUÇÃO

1.1 Problema de pesquisa

O câncer de colo uterino (CCU) é uma doença causada pela infecção recorrente pelo papilomavírus humano (HPV), principalmente pelos tipos graves 16 e 18. O HPV pode ser transmitido por relação sexual, na qual a maioria das pessoas elimina a infecção (WHO, 2022). Os HPVs produzem as proteínas *E6* e *E7*, que desativam genes supressores de tumor, como *tp53* e *Rb*, permitindo um crescimento exagerado das células que revestem o colo uterino, o que potencialmente resultará em câncer (Nakagawa *et al.* 2010).

As classificações histológicas mais frequentes do CCU são o carcinoma de células escamosas (CCE), presente em 75% a 95% dos casos, quando o câncer se origina a partir das células da ectocérvice, e o adenocarcinoma (ADC), presente em 10% a 25% dos casos, quando o desenvolvimento se inicia nas células glandulares da endocérvice. No entanto, esses dois tipos podem ocorrer em conjunto, sendo denominados carcinomas adenoescamosos ou carcinomas mistos (Sharma; Munger, 2020). O CCU é uma doença evitável e curável, mas é o quarto câncer mais frequente em mulheres em todo o mundo, o que o torna um grave problema de saúde pública. A dificuldade de prevenção, rastreamento e tratamento resultou em cerca de 604.000 novos casos e 342.000 mortes em países de baixa e média renda (LMIC) em 2020, segundo a Organização Mundial da Saúde (OMS) (He *et al.* 2020).

A falta de acesso a medidas preventivas, diagnósticos em estágios iniciais da doença e, principalmente, a tratamentos adequados são demandas urgentes a serem cumpridas pelos governos. Por este motivo, a OMS estabeleceu como objetivo eliminar o CCU da lista de doenças públicas até 2100. A estratégia global conta com as metas 90-70-90, que devem ser implementadas pelos países em um prazo de sete anos, com conclusão em 2030 (Aalijahan; Ghorbian, 2019). Essas metas propõem que: 90% das meninas estejam completamente vacinadas contra o HPV até os 15 anos; 70% das mulheres sejam rastreadas de forma eficaz aos 35 e 45 anos; 90% das mulheres diagnosticadas com CCU recebam tratamento adequado (Isin; Dalay, 2015).

O prognóstico do CCU está intimamente ligado à taxa de sobrevida relativa em um período de 5 anos. A avaliação dessas taxas destaca a significativa influência do estágio de diagnóstico na perspectiva do paciente. Para o diagnóstico em estágio inicial, as taxas de

sobrevida em 5 anos para o CCU são de 92%. Contudo, quando o câncer se dissemina para tecidos, órgãos ou linfonodos regionais, essa taxa reduz para 59%. Ainda mais desafiador é o cenário de metástase, onde a taxa de sobrevida em 5 anos diminui substancialmente, atingindo 17% (Fabbri, 2019). Desde 2012, as taxas de incidência do CCU têm diminuído 11% ao ano entre grupos de mulheres de 20 a 24 anos, refletindo os resultados da vacinação contra o HPV. Esse declínio, também observado na mortalidade, reflete os efeitos da prevenção e triagem. Apesar desses avanços, é importante notar que o declínio dessas taxas ainda ocorre de forma gradual (Joshi; Rajender, 2020). No contexto do câncer avançado ou recorrente, o prognóstico frequentemente se torna desfavorável, resultando em baixas taxas de sobrevida, variando de 10% a 20% em um período de 1 ano (Fabbri, 2019).

A abordagem terapêutica padrão para esses casos é a quimiorradioterapia com a cisplatina, um agente anticancerígeno que atua como radiosensibilizador (Zhu *et al.* 2016). No entanto, alguns pacientes podem apresentar resistência adquirida ou intrínseca a esse composto. Esta resistência pode ser atribuída a uma série de fatores, como a redução do acúmulo intracelular dos compostos de platina, o aumento na reparação de danos no DNA e a inativação do processo de apoptose. Além disso, processos como a ativação da transição epitelial-mesenquimal (EMT), alterações na metilação do DNA, perfil de miRNAs, características de células-tronco cancerígenas e a expressão de chaperonas de resposta ao estresse, também podem contribuir para essa resistência (Bhattacharjee *et al.* 2022; Oh *et al.* 2023).

Embora a vacina contra o HPV seja eficaz, o câncer de colo uterino ainda representa um problema de saúde pública global devido à falta de acesso à vacinação e à falta de informação sobre ela. Alguns países enfrentam dificuldades para alcançar certos grupos populacionais, geralmente os mais pobres e distantes dos centros onde ocorrem as campanhas. Além disso, o período de incubação do vírus é longo, o que dificulta a visualização do impacto da vacinação na redução do CCU. Nem todos os países possuem exames para prevenção e triagem, como o exame de Papanicolau, que poderia identificar precocemente o desenvolvimento da doença. As vacinas podem não ser eficazes em casos de exposição a outras cepas do HPV ou em casos de indivíduos imunossuprimidos. Portanto, são necessárias outras abordagens de prevenção, diagnóstico e tratamento para o CCU, por meio de métodos acessíveis, sensíveis e não invasivos (WHO, 2022; Hu; Ma, 2018; NCI, 2021).

1.2 Hipóteses

Os *long non codings RNAs* (lncRNAs) desempenham papéis fundamentais para os mecanismos de resposta à quimiorradioterapia no CCU. Sua expressão diferencial influencia diretamente os processos biológicos subjacentes à progressão do CCU, através de interações complexas com biomoléculas específicas, contribuindo assim para a modulação e potencial inibição da progressão da doença.

1.3 Objetivos

1.3.1 Objetivo geral:

Identificar lncRNAs com expressão diferencial em pacientes com CCU visando sua aplicabilidade como potenciais biomarcadores, e mapear o perfil regulatório associado à resposta à quimiorradioterapia, além de avaliar as interações desses lncRNAs com biomoléculas específicas a fim de elucidar sua contribuição na modulação da resposta terapêutica

1.3.2 Objetivos específicos:

- Analisar o perfil de expressão dos lncRNAs identificados nas amostras de células não-tronco avaliadas.
- Analisar o perfil regulatório dos lncRNAs nos grupos de pacientes respondedoras (R) e não respondedoras (NR).
- Analisar os potenciais impactos dos perfis de expressão gênica nos mecanismos de ação envolvidos, das moléculas de interação e seus alvos, bem como a avaliação de sua contribuição no desenvolvimento, progressão e resistência da doença.
- Selecionar lncRNAs como potenciais biomarcadores que possam ser aplicados na clínica para melhorar o diagnóstico e o tratamento do CCU.

1.4 Justificativa

O diagnóstico tardio do CCU está intrinsecamente relacionado a uma diminuição nas taxas de sobrevivência. A estratégia global delineada pela Organização Mundial da Saúde (OMS), conhecida como "90-70-90", visa avançar na vacinação contra o HPV, no rastreamento e no

tratamento adequado da doença, porém, existem desafios significativos que ainda precisam ser superados para alcançar integralmente esses objetivos (Aalijahan; Ghorbian, 2019). A abordagem terapêutica para o CCU varia de acordo com o estágio da doença, podendo ser limitada à radioterapia ou incluir uma histerectomia prévia. A identificação do estágio da doença é feita através do estadiamento FIGO (Federação Internacional de Ginecologia e Obstetrícia), um sistema que avalia extensão do CCU. Nos estágios mais avançados, a prática recomendada é a cirurgia seguida de quimiorradioterapia concomitante, uma combinação de radioterapia e quimioterapia (Bhatla et al., 2021). Mesmo adotando o tratamento padrão, pacientes em estágios avançados, tais como IB-IIA e IIIB-IVA, enfrentam um risco elevado de recorrência do CCU, com taxas que podem alcançar 70% (Chao et al., 2020). Essa recorrência pode manifestar-se tanto localmente, atingindo o assoalho pélvico ou linfonodos, quanto de forma metastática, afetando órgãos distantes como pulmões, fígado ou ossos (Miccò et al., 2022; Cancer Research UK, 2020).

As investigações destinadas à identificação de novos biomarcadores têm o objetivo de aprimorar não apenas a detecção precoce, mas também o monitoramento após o tratamento do CCU, por meio da exploração de uma diversidade de biomoléculas. Estas incluem desde genes que codificam proteínas até aqueles que não exercem tal função. Pesquisas revelam que numerosos lncRNAs exibem perfis de expressão alterados no CCU, indicando sua possível implicação em uma vasta gama de processos biológicos, tais como crescimento celular, diferenciação, migração, invasão e apoptose. Dentre os lncRNAs investigados, destacam-se o HOTAIR, MALAT1, GAS5 e MEG3, os quais foram associados significativamente a diversos processos patológicos, incluindo a progressão tumoral, a capacidade de invasão e a resistência aos tratamentos existentes (Tornesello et al., 2020; Begliarzade et al., 2023).

Portanto, a investigação da expressão dos lncRNAs visa não apenas aprimorar as estratégias de triagens clínicas, possibilitando diagnósticos precoces e prognósticos mais precisos, mas também desempenhar um papel fundamental no avanço das terapias personalizadas. Além disso, busca-se estabelecer uma correlação entre os genes diferencialmente expressos (DEGs) e a sensibilidade à quimiorradioterapia, contribuindo significativamente para a otimização das abordagens terapêuticas no carcinoma cervical.

2 REFERENCIAL TEÓRICO

2.1 Câncer de colo uterino

Segundo a Organização Mundial da Saúde (2022), o CCU é o quarto câncer que mais afeta mulheres ao redor do mundo, com uma média de 604.000 novos casos (Tab. 1) e 342.000 mortes em 2020 (Tab. 2) (WHO, 2022). São cerca de 13,3 casos por 100.000 mulheres/ano (IC de 95% 13,3 a 13,3) e 7,2 mortes por 100.000 mulheres/ano (95% CI 7,2–7,3) (Chan; Hafiz; Khaw, 2023). A incidência do CCU está fortemente relacionada a fatores socioeconômicos, como renda, educação e saúde, medidos pelo índice de desenvolvimento humano (IDH), aumentando à medida que o IDH diminui (Singh; Vignat; Lorenzoni, 2022). A falta de acesso a medidas preventivas, a diagnósticos no estágio inicial da doença e, principalmente, a tratamentos apropriados corroboram para a evolução da doença. O acompanhamento médico durante a vida das mulheres é a principal ferramenta contra o avanço do CCU, se acessível globalmente, os números de mortalidade seriam significativamente menores (WHO, 2022).

Tabela 1: Incidência global de câncer do colo uterino em 2020.

Classificação	País	Número	TPI/100,000
	<i>Mundo</i>	<i>604,127</i>	<i>13.3</i>
1	Essuatíni	341	84.5
2	Malawi	4,145	67.9
3	Zâmbia	3,161	65.5
4	Tanzânia	10,241	62.5
5	Zimbábue	3,043	61.7
6	Lesoto	541	56.8
7	Uganda	6,959	56.2
8	Comores	167	56.0
9	Moçambique	5,325	50.2
10	Guiné	2,068	50.1

*TPI: Taxas Padronizadas por Idade.

FONTE: Traduzido e adaptado de WCRF (2022). Essuatíni apresenta a taxa de incidência de CCU mais elevada em 2020, seguido pelo Malawi.

Tabela 2: Mortalidade global por câncer do colo uterino em 2020.

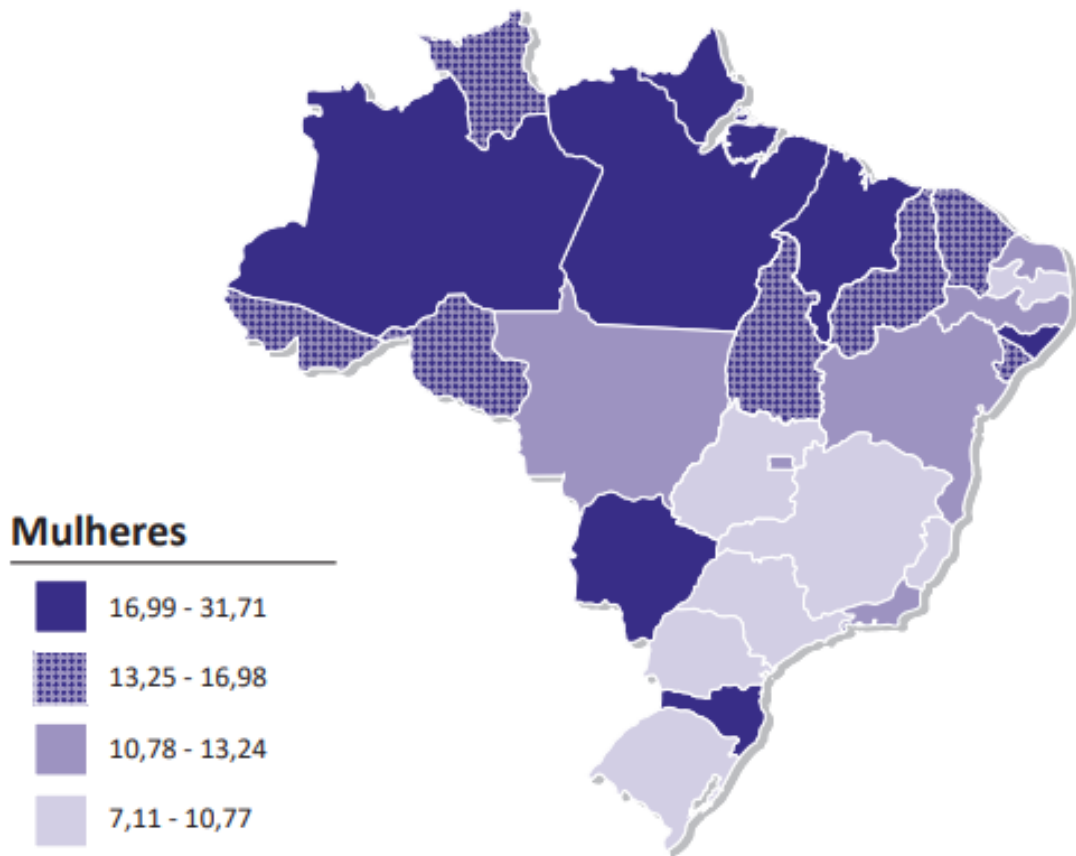
Classificação	País	Número	TPI/100,000
	<i>Mundo</i>	<i>341,831</i>	<i>7.3</i>
1	Essuatíni	214	55.7
2	Malaui	2,905	51.5
3	Zâmbia	1,904	43.4
4	Zimbábue	1,976	43.0
5	Tanzânia	6,525	42.7
6	Uganda	4,607	41.4
7	Comores	109	39.8
8	Moçambique	3,850	38.7
9	Lesoto	362	38.7
10	Burundi	1,126	38.5

*TPI: Taxas Padronizadas por Idade.

FONTE: Traduzido e adaptado de WCRF (2022). Essuatíni apresenta a taxa de mortalidade por CCU mais elevada em 2020, seguido pelo Malaui.

No Brasil, de acordo com o Instituto Nacional de Câncer (INCA), em 2020, houve um total de 6.627 óbitos relacionados ao CCU, representando, em taxa bruta, cerca de 6,12 mortes por cada 100 mil mulheres. No contexto nacional, o CCU é o terceiro tipo de câncer mais comum entre as mulheres, excluindo os casos de tumores de pele não melanoma. Em relação as regiões do país, é o segundo tipo mais comum nas regiões Norte (20,48/100 mil) e Nordeste (17,59/100 mil), enquanto é o terceiro mais prevalente na região Centro-Oeste (16,66/100 mil). Na região Sul encontra-se na quarta posição (14,55/100 mil), e na região Sudeste ocupa a quinta posição (12,93/100 mil) (INCA, 2022a). O INCA também forneceu estimativas para novos casos de CCU para o período de 2023 a 2025 (Figura 1). Para o ano de 2023, foi prevista a ocorrência de 17.010 novos casos, com um risco estimado de 13,25/100 mil mulheres. Essas informações possibilitam uma análise da distribuição geográfica dos casos em nível estadual, incluindo as capitais e o Distrito Federal (INCA, 2022).

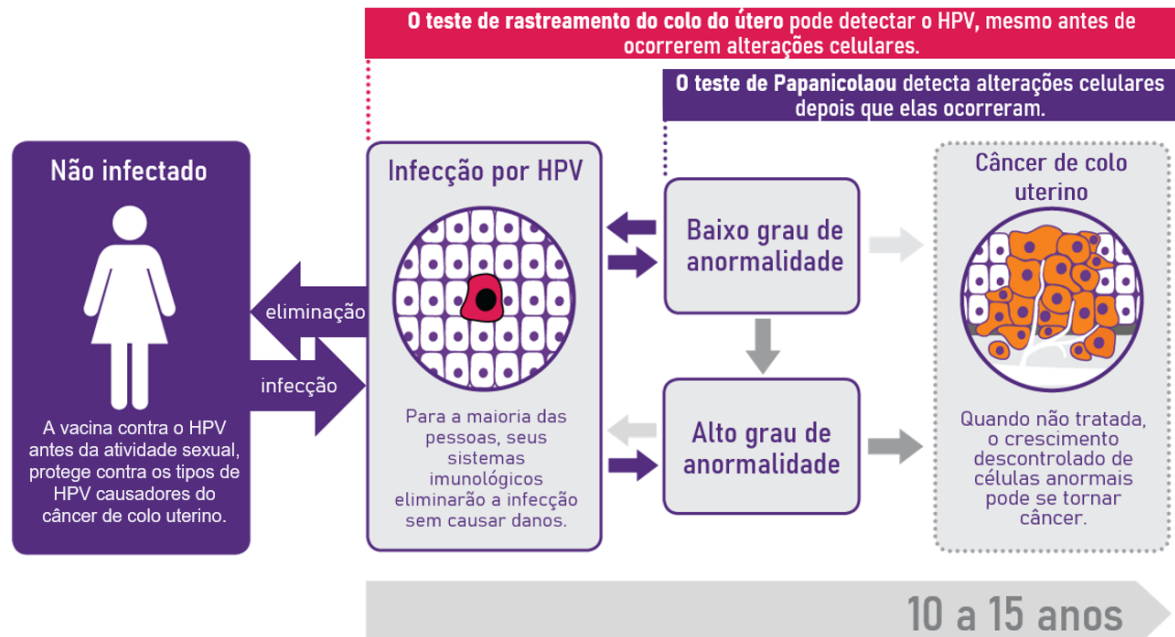
Figura 1: Estimativa de casos de CCU no Brasil para o ano de 2023.



FONTE: INCA (2022). Representação espacial das taxas ajustadas de incidência da neoplasia maligna do colo do útero por 100 mil mulheres, estimadas para o ano de 2023, para o Brasil.

O HPV é o principal promotor desse câncer, presente em aproximadamente 99% dos casos (Vu *et al.*, 2018). Em casos raros, a infecção persistente pelo HPV pode levar ao CCU. São cerca de 10 a 15 anos para que a infecção persistente pelo HPV progrida para câncer (Cancer Institute NSW; 2017) (Figura 2). Os HPVs são uma família de pequenos vírus de DNA dupla fita, não envelopados, os quais são epiteliotrópicos, podendo atingir tratos genitais e respiratórios superiores (Tommasino, 2014; Petca *et al.*, 2020). São divididos como de baixo risco e alto risco. Os de baixo risco, podem causar verrugas nas regiões anogenitais, boca e/ou garganta, como os tipos 6, 11, 42, 43 e 44. Enquanto, o grupo de alto risco tem a capacidade de desencadear vários tipos de câncer, sendo os HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 e 68 os mais incriminados como agentes carcinogênicos (NCI, 2019).

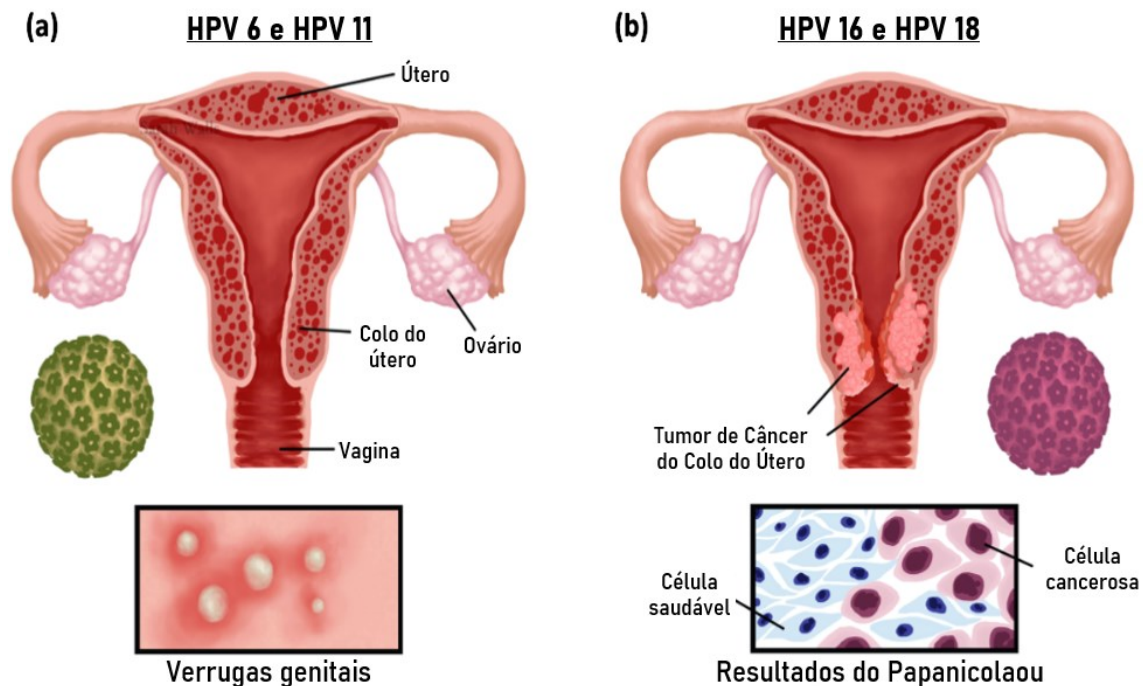
Figura 2: Patogênese do câncer de colo uterino.



FONTE: Traduzido e adaptado de Cancer Institute NSW (2017). Tomar a vacina contra o HPV antes do início da atividade sexual pode proteger contra os principais tipos de HPV relacionados ao CCU. Embora o vírus desapareça naturalmente na maioria das pessoas em 1 ou 2 anos, a infecção persistente pode evoluir para o câncer em um período de 10 a 15 anos. O rastreamento cervical permite a detecção precoce do HPV. Enquanto o teste de Papanicolaou encontra possíveis alterações celulares.

Os principais tipos de HPV relacionados ao CCU são o 16 e o 18, responsáveis por cerca de 70% dos casos. A OMS pré-qualificou quatro vacinas eficazes contra esses tipos, sendo a vacina nonavalente capaz de proteger contra mais cinco tipos de HPV oncogênicos, causadores de mais de 20% dos casos de CCU e outras duas vacinas que atuam contra os tipos 6 e 11 do vírus, que causam verrugas na região anogenital (WHO, 2022) (Figura 3).

Figura 3: Cepas de HPV associadas a verrugas genitais e a carcinogênese cervical.



FONTE: Traduzido e adaptado de Walls (2021). **(a)** As cepas 6 e 11 do HPV normalmente causam verrugas genitais. **(b)** As cepas 16 e 18 do HPV colaboram para que as células cancerígenas se espalhem no colo do útero. As alterações das células do colo de útero são detectadas pelo exame Papanicolaou.

Fatores de risco associados ao CCU, além da infecção pelo HPV, incluem: práticas sexuais sem proteção e com múltiplos parceiros; imunossupressão devido a transplantes ou imunodeficiência; históricos de infecções sexualmente transmissíveis (IST) e/ou de displasias relacionadas ao órgão genital feminino em virtude do HPV; não realizar exames de rastreamento do colo do útero, como o exame citopatológico (Papanicolaou); fatores genéticos; tabagismo (Cohen *et al.*, 2019).

Formas não sexuais de transmissão do vírus podem ocorrer por superfícies contaminadas; transmissão nosocomial; transmissão horizontal através de fômites, pele e dedos em contato com a boca; autoinoculação, o indivíduo contamina seus genitais quando manipula lesões localizadas em outras regiões do corpo; transmissão vertical da mãe para o recém-nascido, através do líquido amniótico infectado, placenta ou durante o parto normal; e a possibilidade pela via aquática, que vem sendo estudada, visto que o HPV está presente na urina e nas fezes (La Rosa, 2016; Petca *et al.*, 2020).

A prevenção primária do HPV é a vacinação, que quando associada à prevenção secundária, por meio de exames citopatológicos, e à prevenção terciária, com identificação e tratamento de cânceres invasivos, tornam o controle da infecção/doença mais abrangentes. Tais estratégias ainda são uma dificuldade para países de baixa e média renda (Vu *et al.*, 2018).

O tratamento preferencial para CCU é a quimioterapia, que conta com o emprego de fármacos anticancerígenos, como a cisplatina, para destruir as células cancerígenas, associada a radioterapia, em que é aplicada a radiação, geralmente raios-x. A administração concomitante desses tratamentos é chamada de quimiorradioterapia, a qual demonstra maior efetividade. Esse tratamento é indicado para casos entre os estágios IB2 e IVA e para pacientes com células cancerígenas nos gânglios linfáticos próximos ao colo do útero após cirurgia (Cancer Research UK, 2020; American Cancer Society, 2021).

2.2 Ferramentas de bioinformática na pesquisa oncológica

Na última década, pesquisadores têm buscado estratégias inovadoras para lidar com o câncer, abrangendo desde o rastreamento até o tratamento. O câncer, sendo a segunda principal causa de morte globalmente (Nagai; Kim, 2017), tem impulsionado a pesquisa oncológica para compreender sua biologia, desenvolvimento e reação a estratégias terapêuticas (Arjmand *et al.*, 2022).

A bioinformática, em particular, possibilita uma análise sistemática do câncer sob diversas perspectivas, como genética, epigenética, redes de sinalização, comportamento celular, manifestações clínicas e epidemiologia (Stransky; Galante, 2009). O advento do sequenciamento de próxima geração (NGS) e projetos dedicados, como o Atlas do Genoma do Câncer (TCGA), confere à aprendizagem de máquina um papel crucial na pesquisa baseada em dados sobre o câncer (Wan *et al.*, 2022).

Dessa forma, a bioinformática tem se estabelecido como uma disciplina essencial na área da saúde, desempenhando um papel de destaque em diversos aspectos. Combinando biologia e tecnologia de informação, a fim de estudar sequências moleculares e dados de ômicas, bem como desenvolver metodologias e ferramentas (NCI, 2019a). Os fundamentos da bioinformática para tratar dados biológicos são: organizar, armazenar, sistematizar, visualizar, anotar, consultar, compreender e interpretar. Suas técnicas computacionais incluem computação em nuvem, estatística, matemática, reconhecimento de padrões, reconstrução,

aprendizado de máquina, simulação e abordagens iterativas e modelagem/algoritmos moleculares (Beg; Parveen, 2021).

A abordagem multiômica, envolvendo genômica, transcriptômica e epigenômica, aliada a métodos computacionais em bioinformática e aprendizado de máquina (ML), tem desempenhado um papel fundamental nesse cenário, ajudando cientistas a desvendar a complexidade da heterogeneidade do câncer, tumorigênese e busca por agentes antineoplásicos e biomarcadores (De Anda-Jáuregui; Hernández-Lemus *et al*, 2020) (Figura 4).

Na medicina, a bioinformática se faz cada vez mais necessária, visto que o sequenciamento completo do genoma humano possibilitou o estudo genético de muitas doenças, como o câncer. Suas contribuições vão desde o desenvolvimento de medicamentos, medicina personalizada, medicina preventiva e terapia genética (Aimst University, 2023). A bioinformática, especialmente na área de câncer, concentra-se na organização e análise de dados para identificar padrões relevantes, com o objetivo último de inovar terapias e abordagens diagnósticas. Um dos aspectos vitais desse campo é o reconhecimento e validação de biomarcadores que não apenas auxiliam na detecção precoce, mas também na avaliação da progressão da doença e na resposta dos pacientes a diferentes modalidades terapêuticas. Isso, por sua vez, tem um impacto significativo na seleção de alvos para medicamentos específicos, tornando os tratamentos mais personalizados e eficazes (Kuznetsov *et al*, 2013; Ahmad; Imran; Ahsan, 2023).

A análise de eventos genéticos associados à doença e a previsão de sua progressão são passos significativos no desenvolvimento de terapias direcionadas para cânceres resistentes a tratamentos convencionais e recorrentes. Os programas de oncologia agora têm sua atenção voltada para entender a resistência aos medicamentos, e isso é possibilitado pelo processamento e análise de dados biológicos, em que os métodos matemáticos e estatísticos computacionais desempenham um papel central (Cao *et al*, 2018; Restrepo *et al*, 2023).

Na pesquisa recente sobre câncer, cientistas têm aplicado métodos inovadores, incluindo rastreamento precoce e estratégias para antecipar resultados de tratamentos. O avanço tecnológico na medicina proporcionou enormes conjuntos de dados sobre o câncer, impulsionando o interesse em prever precisamente os desfechos da doença (Zhang; Shi; Wang, 2023).

A integração da inteligência artificial (IA) na pesquisa sobre câncer está abordando desafios significativos, apresentando resultados promissores. A IA fornece ferramentas e plataformas para melhor compreensão e combate ao câncer. Sistemas baseados em IA

auxiliam patologistas no diagnóstico preciso, reduzindo erros. Modelos de IA preditiva estimam a probabilidade de câncer e identificam fatores de risco (Bindra; Jain, 2023). A combinação de big data e IA capacita especialistas a desenvolver tratamentos personalizados, minimizando efeitos colaterais em comparação com terapias generalizadas (Sebastian; Peter, 2022).

Métodos de aprendizado de máquina tornaram-se essenciais para pesquisadores médicos, identificando padrões em dados complexos e prevendo eficazmente resultados futuros (Garg; Mago, 2021). Com a medicina personalizada em ascensão, foram desenvolvidos estudos que exploram o uso de ML na previsão e prognóstico do câncer. Esses estudos consideram características prognósticas e preditivas, independente ou integradas ao tratamento, orientando a terapia de pacientes (Gaur; Jagtap, 2022). Uma tendência notável é a integração de dados mistos, como clínicos e genômicos. Entretanto, existe uma lacuna comum na validação externa dos modelos propostos (Sigurdsson *et al*, 2023). Apesar disso, a aplicação de ML elevou significativamente a precisão da previsão do câncer em 15% a 20% nos últimos anos (Kourou *et al*, 2015).

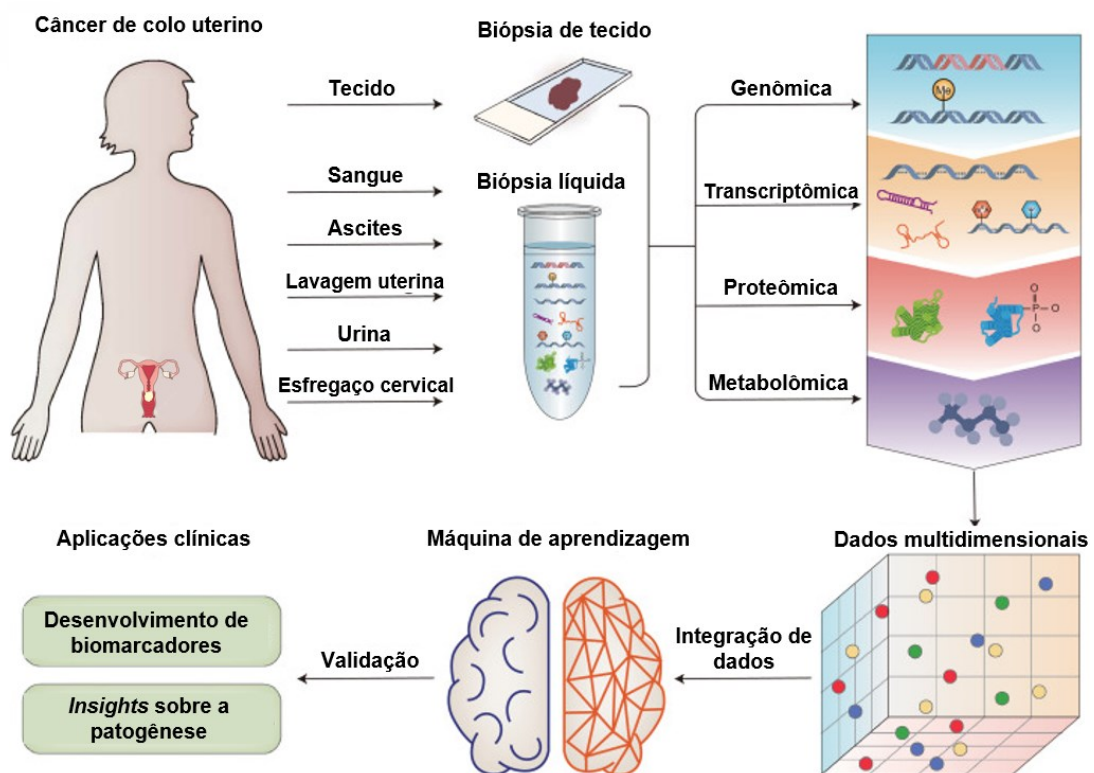
As Máquinas de Vetores de Suporte (SVMs) surgem como uma abordagem recente e promissora no campo de prognóstico do câncer. Essas máquinas otimizam a separação de classes em espaços de características de alta dimensão, permitindo classificações confiáveis (Huang *et al*, 2018). Além disso, SVMs podem fornecer saídas probabilísticas, ampliando sua utilidade (Kourou *et al*, 2015).

Uma das abordagens associadas a aprendizado de máquina, utilizada para auxiliar na avaliação de um conjunto de dados é a árvore de decisão. Na pesquisa oncológica, a análise de árvore de decisão é, muitas vezes, realizada para avaliar o poder preditivo dos potenciais biomarcadores (Rossetto *et al*, 2023). As árvores de decisão são úteis tanto para resolver problemas de classificação quanto de regressão. Recentemente, essas árvores têm sido referidas como Árvores de Classificação e Regressão (CART) (Loh, 2011). O seu funcionamento envolve a construção de uma árvore para analisar uma instância de dados, começando na raiz e se ramificando até chegar às folhas ou nós terminais, onde uma previsão é formulada (Rokach, 2016). A elaboração da árvore de decisão ocorre pela escolha do ponto de divisão mais eficaz para fazer previsões e repetindo o processo até que a árvore atinja uma profundidade pré-determinada (Frank, Hall; Witten, 2016).

No CCU, uma das doenças mais devastadoras que afetam mulheres em todo o mundo, através da tecnologia de sequenciamento de alto rendimento e ferramentas de bioinformática, novas características genéticas e vias de sinalização foram descobertas, oferecendo

perspectivas para um diagnóstico mais precoce e preciso. Essas informações genéticas e vias de sinalização estão agora sendo empregadas para identificar anomalias celulares em estágios iniciais, melhorando os processos de diagnóstico, prognóstico e prevenção de recorrências. Assim, a bioinformática se prova como uma valiosa aliada na área médica, aprimorando a capacidade de entender, detectar e combater doenças complexas como o câncer (Valand *et al*, 2022; Wu; Xi, 2021).

Figura 4: Representação esquemática de abordagens multiômicas para a descoberta de biomarcadores.



FONTE: Traduzido e adaptado de Xiao et al (2022).

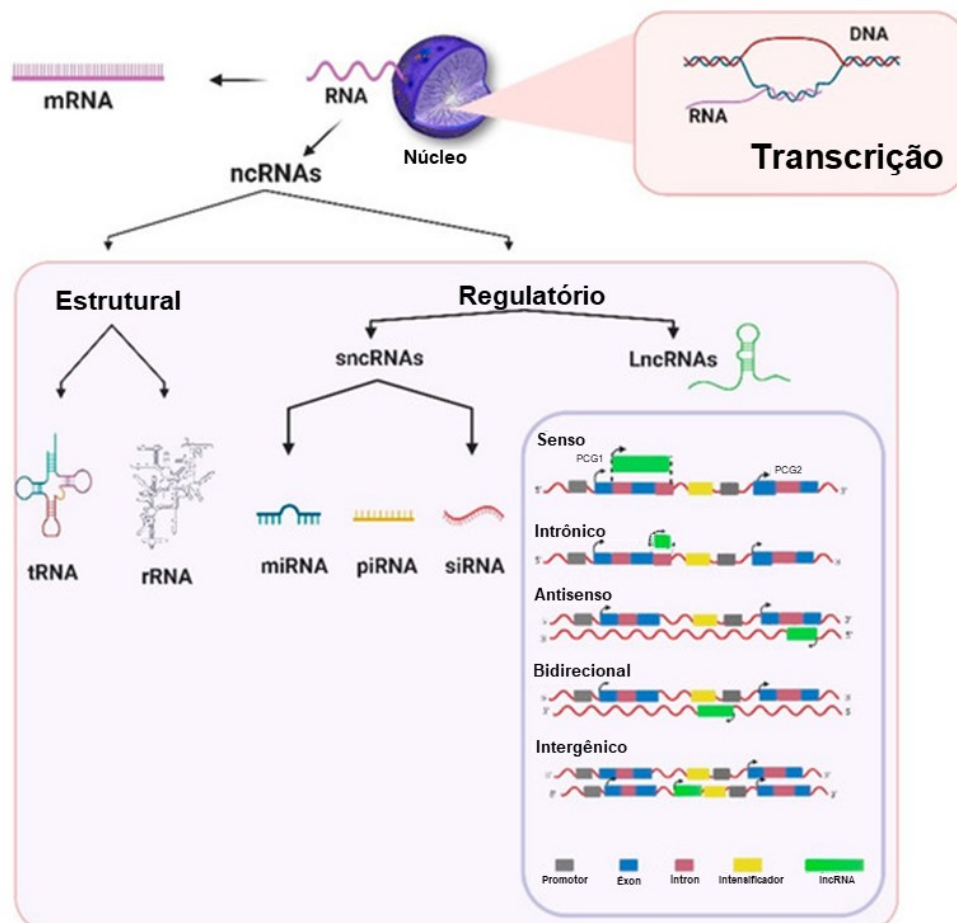
2.3 Long non-coding RNAs

Cerca de 80% do genoma dos mamíferos é transcrito como RNAs não codificantes, enquanto uma pequena porção é transcrita em RNAs mensageiros (mRNAs) codificadores de proteínas, que representam apenas cerca de 2% dos transcritos humanos (Yao; Wang, 2019; Atianand *et al.*, 2017). Por muito tempo, os ncRNAs foram considerados não funcionais, recebendo o título de “região lixo” do DNA. No entanto, avanços nos estudos revelaram sua importância na regulação gênica e ativa participação em doenças. Como resultado, diversos

estudos direcionaram seu foco para esses RNAs buscando compreender e explicar suas funções biológicas (West; Lagos, 2019).

Os ncRNAs são divididos em dois grupos: ncRNAs pequenos (< 200 nucleotídeos (nt)), incluindo tRNA, rRNA, miRNA, snRNA, snoRNA, siRNA e piRNA, e ncRNAs longos (lncRNAs) com > 200 nt de comprimento (Zhang *et al.*, 2017) (Figura 5). Os lncRNAs representam o maior grupo e incluem várias classes de RNAs não traduzidos em proteínas (Ramakrishnaiah *et al.*, 2020). Esses RNAs possuem características únicas, como formas alternativas de biogênese, mecanismos de regulação próprios, domínios funcionais de ligação com DNA, RNA e proteínas, além de padrões conservados e alto grau de especificidade tecidual (Wang *et al.*, 2011).

Figura 5: Classificação dos ncRNAs.

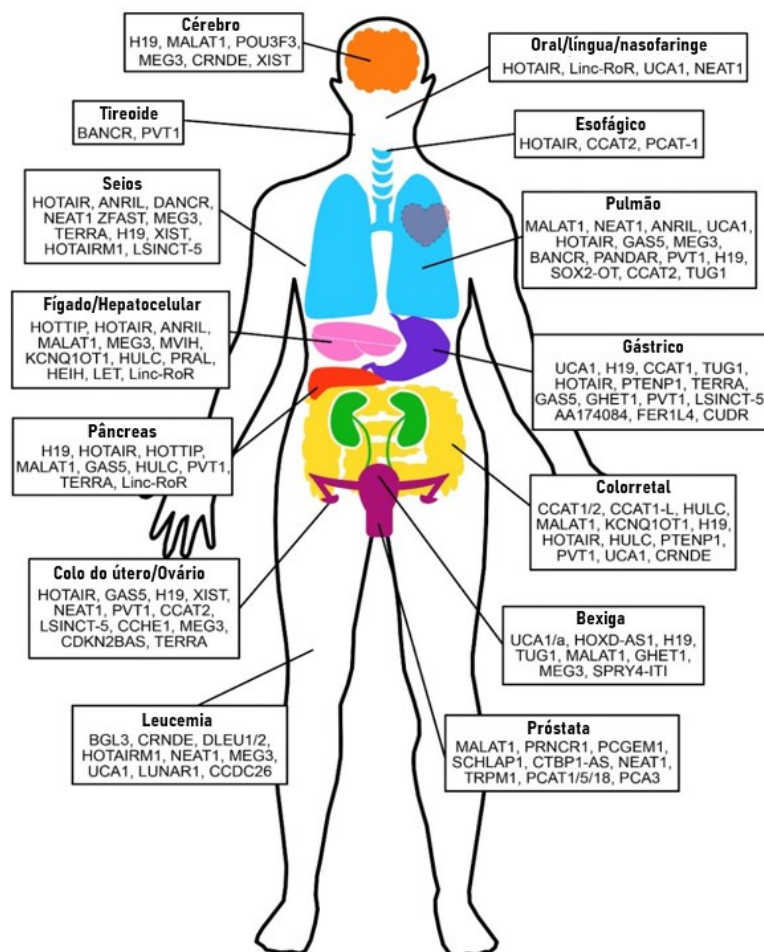


FONTE: Traduzido e adaptado de Ahmad *et al* (2021, p. 02). Os ncRNAs são também classificados em classes estruturais e regulatórias. A classe estrutural inclui rRNA e tRNA. A classe regulatória é dividida em pequenos RNAs não codificantes (sncRNAs), que incluem miRNAs, siRNAs e piRNAs, e os lncRNAs, que são classificados pela sua localização e

aproximação com os genes codificadores de proteínas (PCGs): senso, intrônicos, antisenso, bidirecionais e intergênicos.

Os lncRNAs desempenham um papel fundamental na manutenção da maquinaria de tradução, modulando as funções de outros ncRNAs. Estão envolvidos em diversos processos biológicos, especialmente pelas interações com o DNA, RNA e proteínas. Essas interações permitem que exerçam funções na regulação da expressão gênica, ciclo celular, modificação de histonas e cromatina, metilação do DNA e angiogênese tumoral, bem como o silenciamento de genes. Portanto, mutações e desregulação dos lncRNAs podem interferir diretamente em várias doenças, incluindo as virais, degenerativas e câncer. Além disso, os avanços da transcriptômica e do sequenciamento gênico possibilitaram o conhecimento de uma quantidade significativa de lncRNAs associadas ao câncer (Zhang *et al.*, 2017; Jayasuriya *et al.*, 2022) (Figura 6).

Figura 6: lncRNAs associados a vários tipos de câncer.



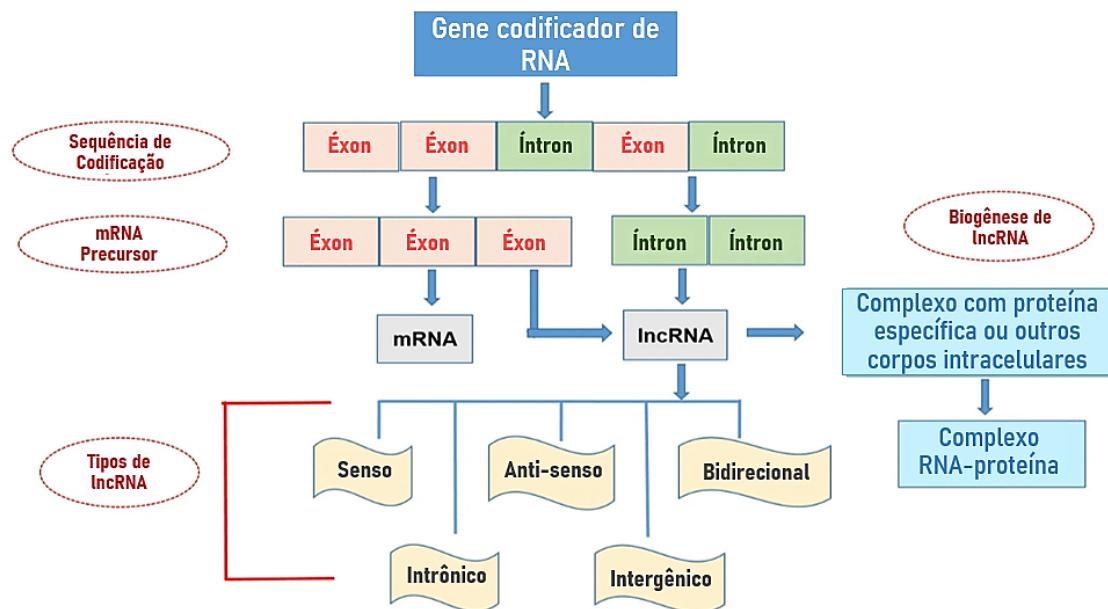
FONTE: Adaptado de Balas; Johnson (2018, p. 111). lncRNAs com expressão aberrante em diferentes tipos de câncer no corpo humano foram documentados.

2.3.1 Biogênese dos lncRNAs

O processo de biogênese dos lncRNAs varia dependendo do tipo celular, estágio e estímulos. A forma de origem não é exclusivamente de seus próprios promotores, mas também de promotores compartilhados com genes codificadores ou não codificadores, transcritos de forma divergente, ou de sequências intensificadoras (*enhancers*) (Statello *et al.*, 2021; Dahariya *et al.*, 2019). Os genes codificadores de lncRNAs apresentam sequências promotoras conservadas, embora com menos motivos de ligação para fatores de transcrição, resultando em níveis de expressão mais baixos.

A regulação da expressão dos lncRNAs ocorre por meio de elementos intensificadores e da interação de potenciadores e promotores, bem como da ligação seletiva de fatores de transcrição específicos do tecido (Choudhuri, 2023). Diferentes classes de lncRNAs são resultantes de elementos do DNA, sendo eles: intensificadores, promotores e regiões intergênicas (Gao *et al.*, 2020) (Figura 7).

Figura 7: Representação esquemática da biogênese de lncRNAs.



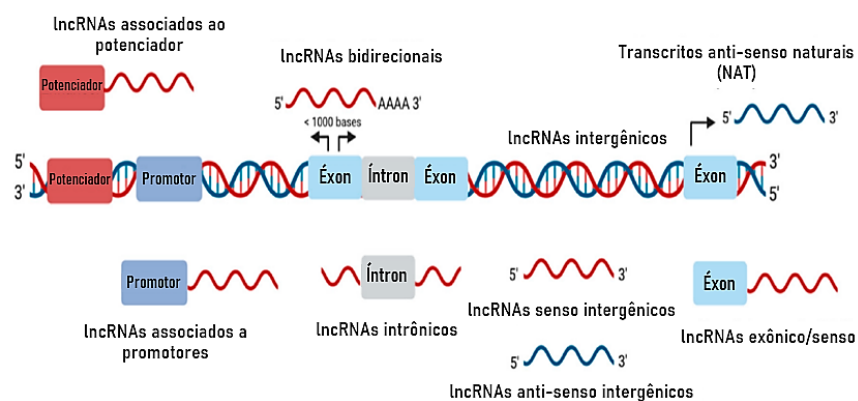
FONTE: Adaptado de Dhanoa *et al.* (2018, p. 03). Os lncRNAs podem ser transcritos a partir de várias regiões do genoma, incluindo íntrons, éxons ou a cadeia oposta de genes codificadores de proteínas. São classificados de acordo com o seu local de origem: sentido (senso), antissenso, intergênico, intrônico e bidirecional. Na biogênese, interagem com

proteínas e estruturas celulares, influenciando sua transcrição e processamento, como RNA polimerase e complexos ribonucleoproteicos (RNP).

As classes de lncRNAs promotores e potenciadores são fundamentais para o controle da regulação gênica. Os potenciadores são regiões genômicas ligadas por fatores de transcrição, que conseguem interagir com promotores, aumentando a expressão gênica. Em alguns casos, podem ser transcritos de regiões a montante do promotor e intensificadores pela RNA polimerase II (Pol II), e outros podem ser transcritos bidirecionalmente de intensificadores também por Pol II. Os lncRNAs intergênicos (lincRNAs), são a subclasse mais estudada, estes são transcritos pela Pol II de regiões entre dois genes (Li *et al.*, 2021; Hou; Zhang; Sun, 2019). Os lincRNAs possuem maior semelhança com os genes codificadores de proteínas em termos de estrutura genômica e comprimento do gene, visto que os lncRNAs que apresentam íntrons (lncRNA nascentes ou pré-lncRNA) sofrem processamento de RNA semelhante aos pré-mRNAs, como *capping*, *splicing* e poliadenilação (Choudhuri, 2023).

Os lncRNAs podem ser classificados com base na sua localização cromossômica. Os lncRNAs senso se sobrepõem a um ou mais éxons de um determinado transcrito na mesma fita, enquanto o antisense (ou transcritos antisense naturais – NATs), utiliza-se do mesmo mecanismo, porém na fita oposta; os intrônicos são transcritos a partir dos íntrons de genes codificadores; os classificados como bidirecionais ou divergentes, são transcrições que compartilham o mesmo promotor com genes codificadores, mas são transcritos na fita oposta; e os lincRNAs possuem *loci* gênicos específicos, se localizam entre dois genes na mesma fita e não apresentam sobreposições (Ma *et al.*, 2013; Joshi; Rajender, 2020) (Figura 8).

Figura 8: Classificação de lncRNAs quanto sua localização cromossômica.



FONTE: Traduzido e adaptado de Lodde *et al.* (2020, p. 02). Os lncRNAs se dividem em cinco categorias baseadas em sua posição: (1) sentido, transcritos na mesma cadeia das proteínas;

(2) antissenso, da cadeia oposta, frequentemente regulam a expressão gênica; (3) bidirecionais, a partir do mesmo promotor, mas em direção oposta; (4) intrônicos, originados de íntrons de genes codificadores de proteínas; (5) intergênicos, transcritos entre genes adjacentes.

Os lncRNAs estão principalmente no núcleo, o que lhes permite desempenhar funções na organização da cromatina, transcrição, processamento de RNA e organização de domínios nucleares. LncRNAs nucleares associados à cromatina são classificados como cheRNAs (RNAs enriquecidos com cromatina) e podem influenciar a arquitetura da cromatina ao interagir com proteínas moduladoras da cromatina (Sun; Hao; Prasanth, 2018). Os lncRNAs são estruturalmente semelhantes aos mRNAs, apresentam um cap 5'-metil-guanosina, uma cauda 3'-poliadenilada e são gerados através da transcrição com RNA polimerase II. Apesar de também sofrerem *splicing* alternativo, nos lncRNAs esse processo ocorre com menor eficiência devido a sinais de *splicing* enfraquecidos e distâncias maiores entre o ponto de ramificação e o local de *splicing* 3' nos íntrons. Quando eficiente, são translocados para o citoplasma, enquanto os que não foram bem processados ficam retidos no núcleo (Choudhuri, 2023).

Os lncRNAs podem interagir com fatores de *splicing* e formar duplex RNA-RNA com pré-mRNAs, afetando a remodelação da cromatina e completando o *splicing* de genes alvo (Dhana et al., 2018). Alguns lncRNAs não poliadenilados, incluindo aqueles associados a regiões intensificadoras, podem ser estabilizados pela clivagem da ribonuclease P (RNase P). Esse mecanismo gera terminações 3' maduras e desenvolvimento de estruturas moleculares circulares, como RNAs circulares (circRNAs) e capeamento por pequenos complexos de ribonucleoproteínas nucleolares (snoRNP) (Shah et al., 2022).

A localização dos lncRNAs no núcleo pode ser coordenada pelos motivos de sequência em cis e fatores em trans. Se transcritos com um ou poucos éxons, os lncRNAs são exportados para o citoplasma através do fator de exportação de RNA nuclear 1 (*NXF1*). A localização desses RNAs no citoplasma, os garantem boa interação com proteínas de ligação a RNAs (RBPs), responsáveis por muitos processos biológicos (Statello et al., 2021). A transcrição por vezes é ineficiente no lncRNA, de modo que ele fique retido no núcleo para ser degradado pelo exossomo nuclear (Jayasuriya, 2022; Dahariya et al., 2019).

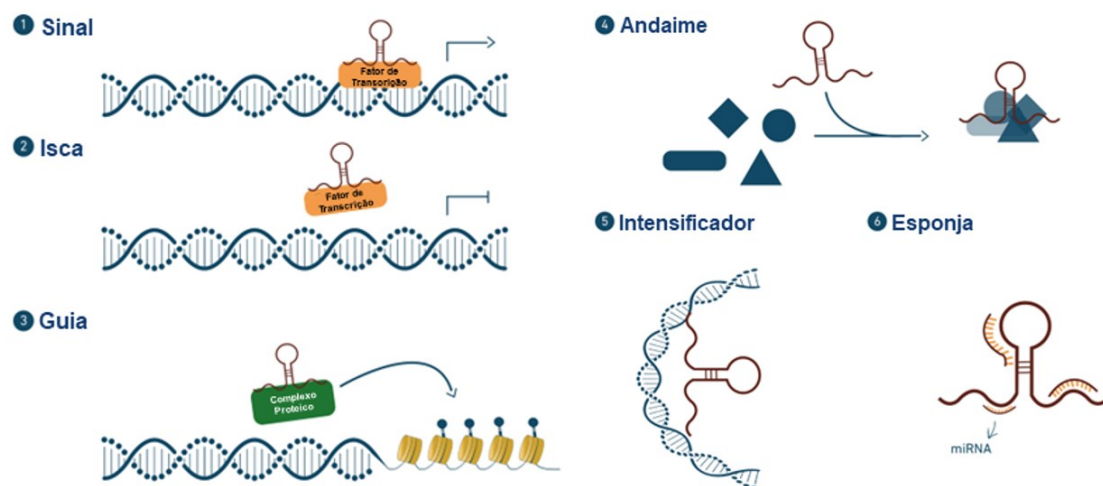
Durante o processo de biogênese de lncRNAs, estruturas subnucleares chamadas de *paraspeckle* (PSs) foram descobertas no lncRNA *NEAT1* (transcrito de montagem de *paraspeckle* nuclear 1). Esses corpos têm funções celulares fundamentais, incluindo respostas

ao estresse celular, diferenciação celular, formação do corpo lúteo e progressão do câncer (Taiana *et al.*, 2020; Wang; Chen, 2020).

2.3.2 Funções dos lncRNAs

Os mecanismos de ação dos lncRNAs determinam suas funções e resultados regulatórios. São classificados funcionalmente pelos mecanismos moleculares que exercem, os quais ocorrem a partir do uso combinado de diferentes arquétipos, chamados de: sinal (*signal*), guia (*guide*), isca (*decoy*), andaime (*scaffold*), potenciador/intensificador (*enhancer*) e esponja (*sponge*) (Figura 9) (Abbas *et al.*, 2020). O mecanismo sinal promove resposta à estímulos, o modo isca a captura de fatores de transcrição/complexos proteicos, enquanto guia direciona fatores de transcrição/complexos proteicos para locais específicos; andaime forma complexos multiproteicos; potenciador/intensificador promove o *looping* cromossômico para aumentar a interação entre regiões intensificadoras e promotoras; e esponja age como um RNA endógeno competitivo (ceRNA) absorvendo miRNAs (Sun *et al.*, 2017; Fang; Fullwood, 2016).

Figura. 9: Modo de ação dos lncRNAs.



FONTE: Traduzido e adaptado de Abbas *et al.* (2020, p. 09). Os lncRNAs desempenham diversas funções, tais como: (1) sinalizando em resposta a estímulos, (2) agindo como iscas que capturam fatores de transcrição ou complexos proteicos, (3) orientando fatores de transcrição ou complexos proteicos para locais específicos, (4) servindo como andaimes para reunir múltiplos complexos proteicos, (5) promovendo o *looping* cromossômico para

intensificar a associação entre regiões intensificadoras e promotoras, e (6) atuando como esponjas de RNA, competindo com miRNAs endógenos.

Muitos lncRNAs podem exercer o papel de sinais moleculares, tanto isoladamente quanto combinados com outras moléculas, como os fatores de transcrição, a fim de promover a transcrição de genes a jusante (Marchese; Raimondi; Huarte, 2017). Essa funcionalidade se justifica pela sua expressão celular específica e a capacidade de responder a vários estímulos. Exemplos deste arquétipo são *XIST* (*X-inactive specific transcript*) e *COLD AIR* (*Cold Assisted Intronic Noncoding RNA*) (Gao *et al.*, 2020).

Outros atuam como guias, auxiliando proteínas específicas a alcançarem sua localização-alvo e exercerem funções biológicas. Interagem frequentemente com fatores de transcrição localizados em sequências específicas de DNA e regulam a transcrição gênica através da forma cis, transcrevendo mRNAs adjacentes, ou em forma trans, controlando a transcrição de mRNAs remotos, em outros *loci* (Schmitz; Grote; Herrmann, 2016; Wapinski; Chang, 2011). Exemplos de lncRNAs guias em forma cis são *AIR ncRNA* e *HOTTIP* (*HOXA Distal Transcript Antisense RNA*) e em trans, *HOTAIR* e *LincRNA-p21* (*Long intergenic noncoding RNA p21*) (Wang *et al.*, 2011).

lncRNAs também podem funcionar como moléculas isca, ligando-se a algumas moléculas de proteínas funcionais para bloqueá-las de regular as moléculas de DNA e mRNA (Quinn; Chang, 2015). Podem ainda se ligar competitivamente aos miRNAs com a finalidade de impedir o efeito inibitório desses sob os mRNAs (Ratti *et al.*, 2020). Exemplos incluem *PANDA* (*P21-associated noncoding RNA DNA damage-activated*) e *DHFR_{ncRNA}* (*Dihydrofolate reductase*) (Wang *et al.*, 2011).

O arquétipo de andaime, exemplificado por *TERC* (*telomerase RNA*) e *HOTAIR*, facilita a interação entre moléculas, possibilitando a montagem de diferentes complexos macromoleculares, promovendo assim a convergência e integração da informação entre diferentes vias de sinalização (Ma *et al.*, 2013; Balas; Johnson, 2018).

Os lncRNAs atuam como potenciadores, influenciando a ativação de genes-alvo. Nesse papel como RNA potencializador (eRNAs), os lncRNAs interagem com a cromatina, modulando as regiões intensificadoras (Hermans-Beijnsberger; Van Bilsen; Schroen, 2018). Essa classe de lncRNAs desempenha um papel crucial na regulação epigenética dos principais elementos da maquinaria de transcrição durante processos celulares associados a doenças. Um exemplo desse arquétipo é o lncRNA *KCNQ1OT1* (*KCNQ1 sobreposto transcrito 1*) (Ahmad; Bensaoud; Mekki, 2021; Tanwar; Reddy; Natarajan, 2021).

A nível molecular, os lncRNAs regulam indiretamente a expressão gênica por meio de sua capacidade de funcionar como "esponjas" moleculares de miRNA, ou seja, como competidores endógenos de RNA (ceRNAs), ligando-se competitivamente e os sequestrando, reduzindo assim a quantidade de miRNAs direcionados a mRNAs (Wang *et al.*, 2021; Yu *et al.*, 2017). Vários lncRNAs não se limitam a apenas um arquétipo, suas características em combinação são fundamentais para o desempenho da função biológica (Wang *et al.*, 2011).

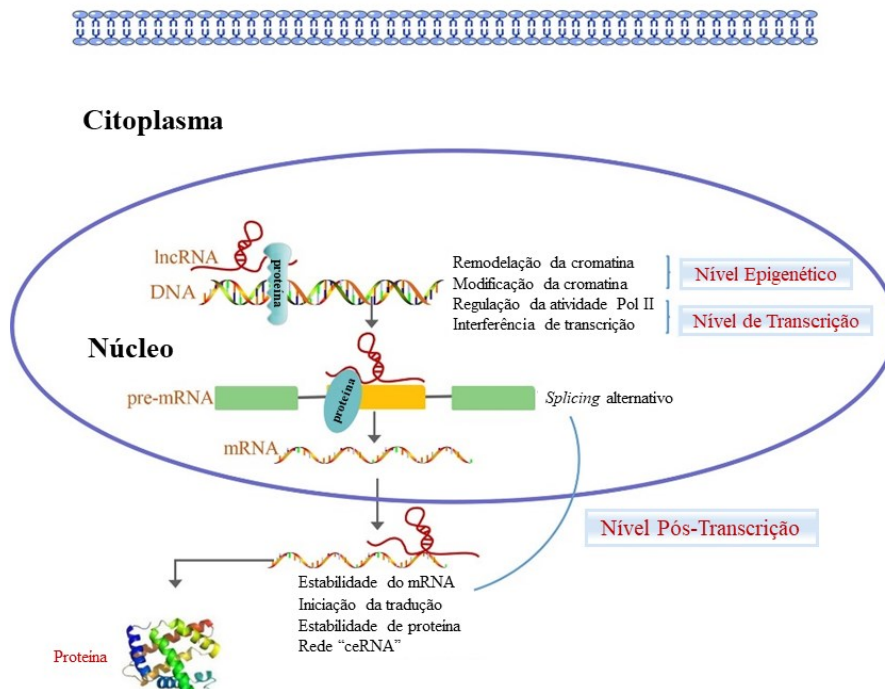
2.3.3 Regulação gênica

Os lncRNAs exercem a regulação da expressão gênica em três níveis: transcricional, pós-transcricional e epigenético, sendo que o modo de ação é influenciado pela localização (Gao *et al.*, 2020; Wu; Liu; Liu; 2014). No núcleo, os lncRNAs desempenham um papel significativo na regulação epigenética, devido à sua variabilidade, flexibilidade e dinâmica genômica. Seus mecanismos regulatórios abrangem o efeito de alosteria, que possibilita a interação com diversas proteínas reguladoras, a habilidade de recrutar proteínas modificadoras da cromatina e o uso de elementos repetidos para modular a expressão de genes próximos (Wang *et al.*, 2017).

Também no núcleo, os lncRNAs são capazes de atuar na autorregulação transcricional, inibindo a transcrição de genes por meio da ligação direta às sequências de DNA, interagindo com proteínas e regulando a expressão de genes a jusante. Eles também funcionam como cofatores, modulando a atividade do fator de transcrição, e exercem um efeito inibitório na transcrição gênica, interagindo com fatores e complexos de transcrição para regular a atividade da RNA Polimerase II (Zhang, X. *et al.*, 2019; Dykes; Emanueli, 2017).

Adicionalmente, estão envolvidos no processo de emenda alternativa do pré-mRNA. No citoplasma, os lncRNAs participam de mecanismos de regulação pós-transcrição, auxiliando na manutenção do *splicing* do pré-mRNA, na estabilidade e tradução dos mRNAs, na estabilidade e atividade proteica, além de atuarem como ceRNAs (Zhang *et al.*, 2017; Gao *et al.*, 2020; Joshi; Rajender, 2020) (Figura 10).

Figura 10: Regulação da expressão gênica dos lncRNAs em três níveis.



Fonte: Traduzido e adaptado de Gao *et al* (2020, p. 04). No núcleo, ocorre o controle do estado epigenético, regulação transcripcional e *splicing* alternativo do pré-mRNA. E, no citoplasma, acontece a regulação pós-transcricional.

2.3.4 Interactoma de lncRNAs

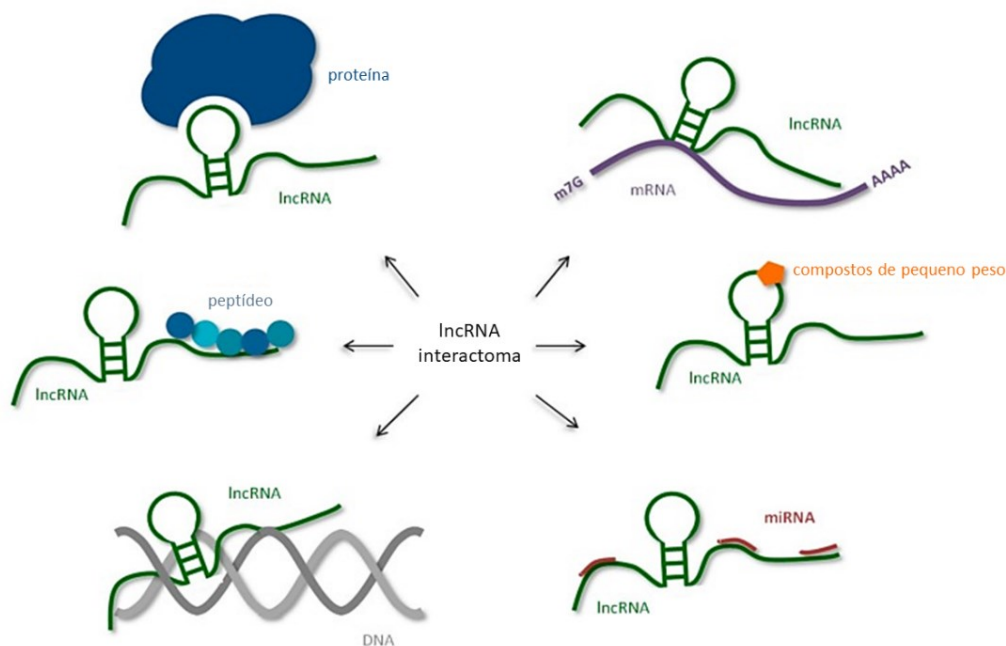
Os lncRNAs podem interagir com várias biomoléculas, como ácidos nucleicos, incluindo miRNAs, mRNAs e DNA, de diferentes maneiras (Figura 11). De um modo geral, na interação com miRNA, os lncRNAs atuam como esponjas ou ceRNAs para miRNAs. Eles possuem sequências complementares aos miRNAs e, portanto, se ligam a eles, impedindo que os miRNAs inibam seus alvos mRNA. Isso resulta em um aumento na expressão dos genes regulados pelos miRNAs (Kazimierczyk *et al*, 2020). Na interação com mRNA, existem alguns mecanismos como (1) interação com maquinaria de metilação: como as maquinarias m7G e a m6A para modular o processamento e estabilidade de mRNAs alvo; (2) modulação da tradução e do *splicing*: os lncRNAs interagem com RBPs envolvidas na maquinaria de tradução e influenciam o *splicing* alternativo de mRNAs, afetando a diversidade de isoformas dessas proteínas produzidas (Sebastian-Delacruz *et al*, 2021).

Na interação com DNA, eles podem agir pela (1) regulação da expressão gênica: interagem com DNA para modular a expressão gênica, podendo se ligar a regiões específicas do DNA e recrutar proteínas envolvidas na regulação da transcrição; (2) modulação da

estrutura da cromatina: tornam certas regiões do genoma mais ou menos acessíveis à maquinaria de transcrição; (3) marcação epigenética: alguns lncRNAs estão envolvidos na marcação epigenética do DNA, como metilação ou desmetilação (Ranjbar *et al*, 2023; Marchese; Raimondi; Huarte, 2017).

O modo de atuação dos lncRNAs pode variar significativamente dependendo do contexto específico e da sequência de interação entre o lncRNA e as biomoléculas envolvidas.

Figura 11: Interactoma de lncRNA humano.



FONTE: Traduzido de Kazimierczyk *et al* (2020). Os lncRNAs possuem áreas especializadas que lhes permitem ligar-se a peptídeos e interagir com outras moléculas. Essas interações podem alterar sua conformação, o que, por sua vez, afeta sua função, permitindo que desempenhem uma variedade de atividades biológicas.

2.3.5 lncRNAs como ceRNAs no CCU

Os lncRNAs têm se caracterizado como atores-chave na regulação do câncer, como o CCU, por meio do mecanismo intrincado de ceRNAs. Nesse cenário, os lncRNAs desempenham uma função crucial ao competirem com os miRNAs por sítios de ligação, resultando em uma rede complexa de regulação pós-transcricional que impacta múltiplos processos biológicos relacionados à carcinogênese cervical (Niu *et al.*, 2020). MALAT1 é um exemplo de lncRNA que atua como ceRNA no CCU. Esse mecanismo serve para regular o

gene MAT2A, através da esponja do *miR-485-5p* no CCU positivo para HPV16. Podendo atuar como um potencial alvo terapêutico nesse câncer (Tie; Ge, 2021).

A interação entre os lncRNAs e os miRNAs é mediada por sequências complementares presentes nos lncRNAs, que servem como locais de ligação para os miRNAs (Ratti *et al.*, 2020). A ligação dos miRNAs a esses sítios nos lncRNAs leva à sua indisponibilidade para se unirem aos seus alvos regulatórios convencionais, frequentemente representados por mRNAs codificadores de proteínas. Esse fenômeno resulta na liberação dos genes alvo da supressão exercida pelos miRNAs, desencadeando uma expressão ampliada desses genes (Braga *et al.*, 2021).

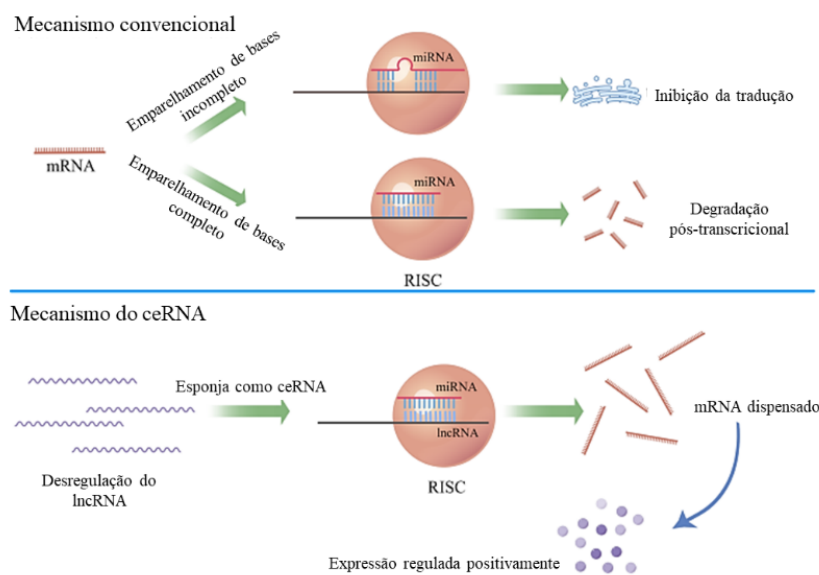
A competição pela ligação aos miRNAs ocorre por meio de sequências parcialmente complementares, também chamadas de "sequências sementes," (ou *seeds*) que consistem em 2 a 8 nucleotídeos. Essas sequências sementes, conhecidas como MREs (Elementos de Resposta a miRNA), desempenham um papel fundamental e indispensável na interação eficiente entre o miRNA e o mRNA. Essas sequências desencadeiam a degradação dos alvos de mRNA ou inibem a tradução a nível pós-transcricional (Yue *et al.*, 2019). No contexto da regulação genética, os lncRNAs também podem competir por MREs com os genes condutores intimamente relacionados à ocorrência e desenvolvimento do câncer, agindo como ceRNAs (Furió-Tarí *et al.*, 2016). Essa complexa rede de interações entre miRNAs, lncRNAs e genes alvo desempenha um papel crucial no processo de câncer, adicionando mais uma camada de complexidade à compreensão da regulação gênica em contextos patológicos (Luan; Wang, 2018) (Figura 12).

A consequência direta da desregulação de miRNA é a alteração da expressão de genes que desempenham papéis fundamentais na progressão tumoral, como iniciação, progressão e metástase. Processos biológicos como proliferação celular, migração, invasão e resistência à apoptose são influenciados por essa regulação cruzada entre lncRNAs, miRNAs e mRNAs (Otmani; Lewalle, 2021; Ala, 2020). A regulação de redes de genes, modulada pelos lncRNAs como ceRNAs, adiciona uma camada de complexidade ao impacto dessa interação nos mecanismos subjacentes ao CCU.

A importância da regulação cruzada mediada por ceRNAs a progressão tumoral do CCU é evidente, considerando-se a interconexão entre os genes alvo dos miRNAs. Diversos genes regulados pelos mesmos miRNAs podem ser modulados pelos lncRNAs, criando uma teia intrincada de interações que amplificam os efeitos dessa regulação pós-transcricional (Ma *et al.*, 2023).

Essa regulação complexa tem implicações terapêuticas promissoras. A manipulação das interações entre lncRNAs, miRNAs e mRNAs é um potencial a ser explorado para desenvolver abordagens terapêuticas direcionadas às vias de genes associados ao CCU. Compreender como os lncRNAs atuam como ceRNAs abre novas perspectivas para o tratamento do CCU, oferecendo um caminho potencial para interromper os mecanismos que impulsionam a progressão tumoral.

Figura 12: Interação entre lncRNAs e miRNAs.



Fonte: Traduzido e adaptado de Xu *et al* (2022, p. 06). No mecanismo tradicional, os RISCs (*RNA-induced silencing complex*) promovem a degradação completa dos mRNAs mediante uma correspondência completa de bases, enquanto a tradução dos mRNAs é impedida pelos RISCs devido a uma correspondência de bases parcial. Entretanto, no mecanismo de ceRNA, os lncRNAs funcionam como "esponjas" de miRNAs, o que resulta na liberação dos mRNAs de sua supressão e, ao mesmo tempo, no aumento da abundância dos produtos expressos.

2.3.6 Envolvimento dos lncRNAs com o câncer

Os lncRNAs desempenham um papel crucial nas vias de sinalização celular relacionadas à iniciação, progressão e disseminação do câncer, influenciando diversos aspectos da formação de tumores, podendo atuar como oncogenes ou supressores de tumores. Esses lncRNAs podem recrutar complexos para remodelação e modificação da cromatina para locais específicos, resultando em alterações na estrutura do cromossomo, além disso

influenciam o status da modificação de histonas e de metilação do DNA. A sinalização celular regulada por esses lncRNAs é um fator importante que contribui para o desenvolvimento do câncer (Peng; Koirala; Mo, 2017; Gao *et al.*, 2020).

A desregulação dos lncRNAs está envolvida na epigenética do câncer, principalmente por meio dos mecanismos de metilação do DNA, alteração do posicionamento do nucleossomo e modificação das histonas, tanto em cis quanto em trans. Através desses mecanismos, esses RNAs podem impactar genes supressores de tumor, recrutando demetilases e acetilases para as regiões promotoras de oncogenes, promovendo a ativação transcricional de genes codificadores de proteínas (Morlando; Fatica, 2018; Isin, M.; Dalay, 2015).

A metilação age no desenvolvimento e na diferenciação celular, devido a isso é um dos principais mecanismos de regulação epigenética, assim, pela sua atuação, é associada à ativação e à repressão da expressão gênica, podendo induzir o silenciamento de um gene (Moore; Le; Fan, 2012). A hipometilação integral do genoma é uma característica do epigenoma canceroso, o que acarreta uma instabilidade genômica (Besselink *et al.*, 2023).

A atuação dos lncRNAs no câncer é fundamental para entender a complexidade dos processos que levam ao desenvolvimento dessa doença. Os lncRNAs possuem diversos papéis oncogênicos e supressores de tumor, isso possibilita sua atuação como biomarcador para diagnóstico (Dhanoa *et al.*, 2018).

O diagnóstico precoce do CCU ainda se mostra um desafio, enquanto a sobrevivência global em estágios avançados e recorrentes permanece preocupantemente baixa, somado a isso, a prevalência da doença está em ascensão entre a população jovem (Burmeister *et al.*, 2022; Schiffman *et al.*, 2016). Embora sejam conhecidos biomarcadores para o CCU, sua especificidade é frequentemente limitada, o que instiga a busca por alternativas de maior sensibilidade e precisão. É nesse cenário que emergem os lncRNAs como promissores marcadores diagnósticos para o CCU, oferecendo um método não invasivo e de execução descomplicada (Arip *et al.*, 2022; Aftab *et al.*, 2021; Onyango *et al.*, 2020).

Muitos lncRNAs apresentam disfunções em diversos tipos de câncer, muitos deles demonstrando estabilidade e detecção viável tanto em tecidos quanto no plasma de pacientes oncológicos (Chi *et al.*, 2019; Dong *et al.*, 2017). Essas perspectivas reforçam o potencial preditivo desses lncRNAs, indicando-os como candidatos viáveis a biomarcadores prognósticos para diversas neoplasias, incluindo o câncer de colo uterino (Jiang *et al.*, 2016).

2.3.7 LncRNAs como biomarcadores

Os lncRNAs são considerados excelentes marcadores moleculares para o CCU, uma vez que desempenham papéis fundamentais em diversos processos biológicos relacionados ao desenvolvimento desse câncer (Peng *et al*, 2015). Sua participação nas atividades de regulação da proliferação celular, apoptose, migração e resistência à quimioterapia e radioterapia os torna moléculas de grande interesse na compreensão da modulação da resposta ao tratamento de quimiorradioterapia (Zhang H. *et al*, 2022).

Estudos já demonstraram que os lncRNAs podem influenciar vias de sinalização relacionadas à radioterapia, desempenhando um papel importante na resposta ao tratamento (Xie *et al*, 2022). Além disso, os lncRNAs são mais específicos em comparação aos mRNAs e estão intimamente relacionados à regulação da expressão gênica.

Por exemplo, Chen Qiantao detectou alterações nos perfis de expressão de lncRNAs em tecidos do CCU e em tecidos normais, utilizando tecnologia de Chip-seq de alto rendimento. Foram identificados um total de 30.586 lncRNAs, dos quais 22.043 apresentaram expressão diferencial. Entre esses, 11.545 estavam regulados positivamente, enquanto 10.498 estavam regulados negativamente (Begliarzade *et al*, 2023).

Para que um lncRNA seja considerado um biomarcador de interesse, é crucial que ele demonstre uma expressão significativamente diferencial entre grupos de pacientes, como as respondedoras e não respondedoras. Além disso, é essencial que haja uma associação entre o lncRNA alvo e os mecanismos biológicos relacionados à doença, a fim de compreender seu papel no contexto do CCU (Beermann *et al*, 2016). O biomarcador deve ser altamente específico para a doença em estudo e suficientemente sensível para detectar variações na progressão da doença, na resposta ao tratamento ou na recorrência, no caso do CCU (Arriaga-Canon *et al*, 2023).

Outro requisito importante é que o lncRNA seja estável e detectável em amostras biológicas coletadas clinicamente, como sangue, urina ou tecidos. Os resultados de detecção também devem ser reprodutíveis em diferentes laboratórios e análises independentes (Badowski; He; Garmire *et al*, 2022). Além disso, é fundamental que o lncRNA seja validado em estudos clínicos de grande escala para confirmar sua utilidade como biomarcador (Hall *et al*, 2017). Por fim, é preferível que os biomarcadores sejam práticos, acessíveis e adequados para uso em um ambiente clínico, respeitando princípios éticos e garantindo a segurança dos pacientes.

CAPÍTULO 2

The upregulation of lncRNA ENSG00000267838 is related to the high risk of progression and non-response to chemoradiotherapy treatment for cervical cancer.

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ABSTRACT

Cervical cancer (CC) is primarily caused by persistent infection with oncogenic types of human papillomavirus (HPV), and is a global public health concern. The World Health Organization (WHO) has established a plan to eliminate CC as a public health issue by the year 2100. However, the implementation of the HPV vaccine is impeded by vaccine restrictions and misinformation, despite its demonstrated effectiveness. The treatment of CC is influenced by disease stage, with unfavorable prognosis for those in advanced or recurrent stages. This study aims to analyze the lncRNAs expression patterns in patients with diverse responses to chemoradiotherapy for cervical cancer and elucidate their regulatory mechanisms and molecular interactions related to the disease development and treatment. Non-stem cells from CC were isolated using flow cytometry sorting and used for total RNA extraction. The

RNA was used to build libraries that were subsequently sequenced using the Illumina Nextseq 550. 417 lncRNAs were identified with differential expression between samples from patients who responded or did not respond to treatment. Further analysis demonstrated that these lncRNAs significantly interact with several biomolecules which play crucial roles in cervical cancer progression and therapeutic resistance. Statistical analysis correlated the expression profile of these lncRNAs with treatment efficacy. Three lncRNAs, ENSG00000267838, ENSG00000266340 and FRMD6-AS1, were identified, and their positive expression is related to non-response to chemoradiotherapy for CC and worse progression-free survival. Specifically, lncRNA ENSG00000267838 has its up-regulation related to non-response and down-regulation to response to chemoradiotherapy treatment.

Keywords: long non-coding RNAs; CC; cervical cancer; gene expression; regulatory profile; biomarkers.

INTRODUCTION

Cervical cancer (CC) is a preventable and curable disease and is the fourth most common cancer in women worldwide. In 2020, there will be approximately 604,000 new cases and 342,000 deaths in low- and middle-income countries (LMIC), according to a World Health Organization (WHO) report [1]. The most common histologic forms of CC include squamous cell carcinoma (SCC) in 75-95% of cases and adenocarcinoma (ADC) in 10-25% of cases [2].

CC is caused by persistent infection with human papillomavirus (HPV), primarily types 16 and 18 [3]. These viruses produce the E6 and E7 proteins, which inactivate tumor suppressor genes such as tp53 and Rb [4]. The WHO has set the goal of eliminating cervical cancer as a public health problem by 2100 through the global 90-70-90 strategy, which aims to vaccinate 90% of girls by the age of 15, screen 70% of women between the ages of 35 and 45, and provide appropriate treatment for 90% of those diagnosed by 2030 [5, 6].

The prognosis of CC is closely related to the 5-year relative survival rate. Evaluation of these rates highlights the significant impact of the stage of diagnosis on the patient's outlook [7]. When diagnosed early, the 5-year survival rate for CC is 92%. However, once the cancer has spread to regional tissues, organs, or lymph nodes, this rate drops to 59%. Even more challenging is the metastatic scenario, where the 5-year survival rate drops significantly to 17% [8, 9].

Despite advances in vaccination and screening, the decline in incidence rates is gradual [10]. Vaccines may not be effective in individuals exposed to different strains of HPV or in immunosuppressed individuals. Therefore, additional approaches are needed to prevent, diagnose, and treat CC using accessible, sensitive, and noninvasive methods [3, 11, 12].

The main therapeutic approach is chemoradiotherapy with cisplatin, an anticancer and radiosensitizing agent [13]. The combination of cisplatin and paclitaxel is also a common alternative in the treatment of CC, with response rates ranging from 29.1% to 67%. However, resistance to cisplatin, whether intrinsic or acquired, is a significant challenge that compromises treatment efficacy [14]. Processes such as epithelial-mesenchymal transition (EMT), changes in DNA methylation, miRNA profiling, cancer stem cell characteristics, and expression of stress response chaperones may contribute to cisplatin resistance [15, 16].

Integrating biomarkers into CC screening is challenging because the lack of a uniform scoring system hinders the consistent application of techniques such as immunohistochemistry and cytology [17]. Biomarker discovery studies aim to improve CC detection and post-treatment monitoring. Long non-coding RNAs (lncRNAs) are characterized by differential expression, suggesting involvement in processes such as cell growth, differentiation, migration, invasion, and apoptosis [18].

The role of lncRNAs in cancer is closely related to their expression profile and their ability to interact with proteins, mRNAs, and miRNAs. In the case of CC, resistance to antineoplastic drugs is a limitation in chemoradiotherapy treatment. For example, the lncRNA PVT1 is associated with resistance to cisplatin and paclitaxel and promotes epithelial-mesenchymal transition (EMT) in CC. Low expression of CASC2 contributes to cisplatin resistance, while PTEN may increase chemosensitivity. High NEAT1 expression correlates with resistance to radiotherapy [18, 19].

This study aims to fill a critical gap in the understanding of CC by focusing on cervical cancer non-stem cells (CCNSCs). The identification of lncRNA expression profiles linked to therapeutic response in CC presents a promising opportunity for discovering biomarkers that can be accessed through liquid biopsy, both for diagnosis and monitoring treatment efficacy. This approach has the potential to improve the speed and effectiveness of treatment, enabling the implementation of more personalized medicine that can substantially influence survival outcomes, which are notoriously challenging for patients with CC. In addition, considering quality of life during survival is undeniable, highlighting the importance not only of survival, but also of patients well-being after treatment. This pipeline, described in detail in the material and methods section, enabled a systematic assessment of molecular interactions and gene expression in response to treatment (Figure 1).

MATERIAL AND METHODS

Patient Recruitment and Sample Collection

This study collected samples from 31 patients with CC at the Mario Penna Institute in Belo Horizonte, Brazil, between August 2017 and May 2019. Ethical standards were followed with the approval of the local research ethics committee. Inclusion criteria included no history of cancer or immunologic disease, histopathologic diagnosis of adenocarcinoma or squamous cell carcinoma, FIGO stage II or III at diagnosis, and signed informed consent. Approval was granted by the local Institutional Review Board (approval number 1.583.784) [20].

Treatment and patient staging

After diagnosis and biopsy, the patients underwent chemoradiotherapy, and all clinical data was meticulously collected and analyzed. The patients selected had a median age of 50 years. The classification of these patients was based on two main criteria: response (R) and non-response (NR) to treatment. Patients were classified as "R" if there was no evidence of cervical neoplastic lesions for a period of up to 8 months after chemoradiation. In contrast, patients were classified as "NR" if there was persistence of cervical neoplastic lesions, including partial response, tumor progression, or disease stabilization during the same period. At the end of the classification, a total of 21 patients were classified as R and 10 as NR [20].

Sequencing libraries preparation

Fluorescence-activated cell sorting (FACS) was used to isolate cervical cancer stem cells (CCSCs) and cervical cancer non-stem cells (CCNSCs) from a complex mixture of tumor cells based on their light scattering and fluorescence staining profile, as described by Zuccherato *et al.* (2021). The selected cells were processed for complementary DNA (cDNA) synthesis using the SMART-Seq v4 ultra-low input RNA sequencing kit (Takara Bio USA, CA). RNA sequencing library preparation was performed using the Nextera® XT Library Prep Kit (Illumina™) with the Nextera® XT Index Kit V2 Set A (Illumina™) according to the manufacturer's instructions. Sequencing of the libraries was performed using the Nextseq 550 (Illumina, CA). Sixty-two libraries with more than 20 million reads each were analyzed. The complete methodology, and subsequent sequencing, including casuistry, RNA

preparation steps, and subsequent sequencing, is available in the article by Zuccherato *et al.*, 2021 [20]. In this study, only the sequences related to the non-stem portion were analyzed.

Data Preparation

For the sequence quality analysis, the FastQ files containing the sequences were evaluated using the FastQC program (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). The Cutadapt tool [21] was used to remove low-quality sequences with a Phred score ≤ 25 at the 3' end of the reads and adaptors. The integrity of the sequences was then re-assessed using FastQC.

STAR software (<https://github.com/alexdobin/STAR>) was used to map and align the clean sequences to the human genome (Homo sapiens GRCh38). The parameters "quantMode GeneCounts" and "outFilterMultimapNmax 1", based on the GRCh38 and GTF files of the reference human genome obtained from the GENCODE database, were used to quantify the transcripts that aligned exclusively to a specific gene. Genes with counts less than ten were disregarded. The FASTA sequences of the obtained IDs were retrieved from the nucleotide sequence file of lncRNA transcripts in the reference chromosomes, version 40, from GENCODE (<https://www.genencodegenes.org/human/>). The sequences are available in the SRA database (PRJNA812529).

Analysis of differentially expressed genes

DEGs were visualized on heat maps using the Pheatmap package in R (<https://www.rdocumentation.org/packages/pheatmap/versions/1.0.12/topics/pheatmap>). For analysis, only DEGs with a $\log_2FC > 1$ or < -1 and an adjusted p-value (p_{adj}) < 0.05 were considered. These DEGs were utilized as targets in ClusterProfiler [22] to explore their biological functions. Through this package, gene enrichment analysis was conducted to identify pertinent biological process (BP) terms associated with the set of DEGs.

Analysis of the functional roles of lncRNAs

The AnnoLnc2 program [23] was utilized for the systematic annotation of lncRNAs, enabling a comprehensive functional study, including expression patterns, co-expression analysis in normal and cancer tissues, regulation, interactions with biomolecules, genetic

associations, and evolution. To comprehend the biomolecules associated with these transcripts, literature searches and database queries were conducted using Human Protein Atlas - HPA (<https://www.proteinatlas.org/>), Genotype-Tissue Expression - GTEx (<https://www.gtexportal.org/home/>), Universal Protein - UniProt (<https://www.uniprot.org/>), BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), and UCSC (University of California at Santa Cruz) Genome Browser (<https://genome.ucsc.edu/>) databases.

Decision Tree Analysis for DEGs

We evaluated a total of 18,805 lncRNAs using the annotation file, which contains nucleotide sequences of lncRNAs on the human reference chromosomes (GRCh38 v.40). Subsequently, the identified DEGs were analyzed through the AnnoLnc2 software. This analysis considered the transcripts' expression in female tissues and their relevance to CC.

The C4.5 algorithm of WEKA software (Waikato Environment for Knowledge Analysis, version 3.6.11, University of Waikato, New Zealand) was implemented with the parameters of the J4.8 algorithm [24, 25] to construct the decision tree. The dataset consisted of 31 records, divided into two groups: 21 R (67.74%) and 10 NR (32.26%) records. For classification, the entire dataset (FULL) was used to develop and train the classification algorithms. Leave-one-out cross-validation (LOOCV) was used to estimate the performance of the classification algorithms.

Survival and Risk Analysis

To evaluate a possible association between the expression profile of DEGs and progression-free survival, Kaplan-Meier (log-rank) analyses were performed using SPSS software (version 20, IBM, USA). Cox regression analysis was used to quantify the impact of this association. The R survival and coxphf packages (<https://cran.r-project.org/web/packages/>) were used. The hazard ratio (HR) values obtained were visualized in a forest plot generated in GraphPad Prism 9 (GraphPad Software, USA). Only results with a p-value < 0.05 were considered statistically significant. The sensitivity and specificity analysis was performed using WEKA software.

RESULTS

Histopathologic Characteristics

In this study, patients were classified based on the histological subtype of cervical cancer, with squamous cell carcinoma being identified in 30 patients and adenocarcinoma in one patient. The analysis then concentrated on the response to chemoradiotherapy, categorizing patients into responders (R, 21 cases) and non-responders (NR, 10 cases). Notably, significant parametrial involvement was evident, with bilateral involvement in 68% of the cases and unilateral involvement in 29%. Only one patient, constituting 3% of the sample, exhibited no parametrial involvement. In terms of histopathologic grading, Grade II was predominant, observed in 61% of patients, while Grade III was identified in the remaining 39%. As for FIGO stage distribution, the results were as follows: Stage IIA in one patient (3%), Stage IIB in 42% of cases, and Stage IIIB being the most prevalent, affecting 55% of the cohort.

Differential Expression Profile Analysis

In the comparative analysis between the responder and non-responder groups, 417 out of 18,805 lncRNAs annotated in GENCODE version 40 were identified as differentially expressed genes. This variance in gene expression profiles between the groups indicates a potential link between lncRNA expression and the response to treatment in cervical cancer. A noteworthy observation was the identification of a reciprocal expression pattern, wherein genes upregulated in the NR group were downregulated in the R group, and vice versa (Figure 2). The investigation then concentrated on examining the expression profiles of these differentially expressed lncRNAs in non-stem cells derived from both R and NR groups. The focus on these specific lncRNAs aimed to elucidate their role in influencing the outcome of the treatment.

Gene Ontology Analysis

In the analysis, only genes that demonstrated significant enrichment in Gene Ontology (GO) terms, with an adjusted p-value (padj) of less than 0.05, were taken into consideration. From the total of 30,065 genes categorized under the biological process (BP) category, 18,505 genes constituted the background list, and 417 differentially expressed genes comprised the target list for this analysis. Within this category, five genes—SNHG29, CDKN2B-AS1, NEAT1, MALAT1, and SCARNA9—were highlighted. Notably, the latter four genes have been previously documented in the scientific literature for their roles in cervical cancer, underscoring their potential significance in the disease's pathophysiology.

Systematic Analysis of DEGs

The comprehensive examination of the 417 differentially expressed genes through the AnnoLnc2 software enabled an in-depth understanding of the roles that the studied lncRNAs may play. This analysis encompassed a variety of factors, including the potential for coding, genomic positioning, secondary structural characteristics, and expression data. Additionally, it delved into co-expression patterns, subcellular localization, evolutionary history, interactions with proteins and miRNAs, presence of repeat elements, and the regulation of gene transcription. By expanding the scope of investigation to include the entire female reproductive system beyond just cervical tissue, the study discovered that 28 of the 417 DEGs are expressed in normal vaginal tissue, 21 in the ovary, and 19 in the uterus. Further refining the analysis to focus on the interactions between transcripts and biomolecules led to the identification of 8 DEGs that exhibit potential interactions and possess specific regulatory patterns.

DEGs filtering

The AnnoLnc2 software was utilized to sift through the differentially expressed genes to pinpoint transcripts of potential significance to the biological processes implicated in cervical cancer. The initial step involved assessing candidates for their expression across a range of tissues including the endocervix, ectocervix, uterus, ovaries, and vagina, in addition to cell lines (notably HeLa) and cancers affecting the female reproductive system, with an emphasis on CC. This stage of analysis resulted in the identification of 49 DEGs that satisfied these criteria, as detailed in Table 1. Subsequently, the expression profiles of these 49

transcripts were examined, revealing a distinct gene signature capable of distinguishing between the responder and non-responder groups, as illustrated in Figure 3.

Modulation of expression by transcription factors

In the investigation of gene regulation mechanisms, our study delves into the role of long non-coding RNAs in modulating gene expression via interactions with transcription factors (TFs) and their capacity to function as TFs themselves, thereby controlling chromatin remodeling processes [26, 27]. The TFs associated with genes of interest in the context of cervical cancer fulfill a variety of functions ranging from tumor suppression to the facilitation of tumor progression. These activities are pivotal in influencing overall and progression-free survival rates, advanced stages as per the FIGO classification, and the likelihood of cancer recurrence [28 – 30]. Furthermore, their impact extends to critical cellular processes such as senescence, metastasis, and resistance to radiation and chemotherapy, as outlined in Table 2 [31].

Interaction with proteins

Our research identified proteins linked to differentially expressed genes that play a crucial role in the complexity of cervical cancer, as detailed in Table 3. The identification of a high mutational burden within regulatory proteins contributes to the emergence of factors leading to genomic instability in CC [32, 33]. Functioning as RNA-binding proteins, these proteins regulate genetic elements, influencing genes, pathways, and processes such as ferroptosis [34]. Furthermore, we underscored the significant role of these proteins in cell cycle progression, where they act as coactivators of tp53 and are pivotal in the degradation of tp53 mediated by the HPV-16 viral oncoprotein E6. Such interactions are instrumental in altering miRNA profiles that affect cell motility, thereby influencing migration and invasion events [35]. Remarkably, these proteins are related to the progression of tumor size and other clinicopathological features, playing a key role in the dynamics of tumor progression with consequential effects on survival and clinical outcomes [36, 37].

Association with miRNAs

Our analyses have uncovered a pivotal and multifaceted role for miRNAs associated with lncRNAs in the realm of CC, as outlined in Table 4. The miRNAs identified through the AnnoLnc2 tool are instrumental in the regulation of tumor growth and progression, employing complex mechanisms. This includes the interaction with lncRNAs, such as the lncRNA MIR205HG, which functions as a competing endogenous RNA and influences tumor proliferation by sequestering miR-122-5p in CC [38, 39]. Furthermore, these miRNAs are capable of modulating radiosensitivity and chemosensitivity, prompting cell cycle arrest and early apoptosis, and serving as tumor suppressors [40, 41]. Additionally, our findings emphasize the profound connections these miRNAs have with overall survival, tumorigenesis, and migration in CC, where they act as tumor suppressors and augment sensitivity to cisplatin, underscoring their significant potential as therapeutic targets and prognostic markers.

Refinement of the 49 lncRNAs

In our quest to identify lncRNAs of potential relevance to CC, a meticulous refinement strategy was employed. This process involved the analysis of transcript interactions with various biomolecules, prioritizing those interactions that are significant, and underscoring the biological functions executed by these molecules. Through this rigorous refinement process, a subset of 8 DEGs was identified as particularly pertinent: MIR205HG, NORAD, LINC00189, RUSC1-AS1, PITPNA-AS1, CDKN2B-AS1, MIR210HG, and OIP5-AS1. This selection was carefully made with an emphasis on the interactions of these genes with transcription factors, miRNAs, and proteins previously linked to CC. The aim was to pinpoint promising candidates that merit further exploration, as illustrated in Figure 4.

Statistical Analysis for DEGs Selected as Potential Biomarkers

Decision Tree

Decision tree analysis was utilized to discern genes that could serve as biomarkers crucial in determining therapy responses, based on their expression profiles of either positive or negative regulation (upregulation and downregulation) for the R and NR patient groups.

Among the 49 DEGs identified with expression associated with CC, this analysis methodically selected a highly informative subset of three genes: ENSG00000267838, FRMD6-AS1, and ENSG00000266340. The decision tree exposed intricate expression patterns of these genes, signifying their linkage with either a favorable response to treatment or a lack thereof. The accuracy of the decision tree analysis was 80.64% for Leave-One-Out Cross-Validation (LOOCV) and 90.32% for FULL dataset training, as depicted in Figure 5. The analysis indicated that downregulation of ENSG00000267838 and ENSG00000266340 might be associated with a positive treatment response, while upregulation of these genes, along with FRMD6-AS1, could suggest a non-responsive behavior to therapy. A further decision tree focusing on ENSG00000267838 reinforced the association of its downregulation with treatment responsiveness, in contrast, its upregulation was linked to non-responsiveness, achieving classification accuracies of 80.64% (LOOCV) and 87.09% (FULL), as illustrated in Figure 6.

Progression-free survival analysis

Progression-free survival analysis, employing the Kaplan-Meier method, was conducted to ascertain the relationship between the expression of various lncRNAs and adverse outcomes such as recurrence, relapse, or mortality in CC patients. This examination focused on eleven lncRNAs, revealing distinct patterns of association with patient prognosis. Specifically, MIR205HG, NORAD, and PITPNA-AS1 demonstrated statistical significance (p -value < 0.05), linking their lower expression levels to a poorer prognosis. Conversely, ENSG00000267838, FRMD6-AS1, and ENSG00000266340, also achieving statistical significance, were associated with a higher risk when overexpressed, implicating their upregulation in disease progression and underscoring their potential value as prognostic biomarkers. For the remaining lncRNAs, including RUSC1-AS1, OIP5-AS1, MIR210HG, CDKN2B-AS1, and LINC00189, no significant differences in expression profiles were noted, as illustrated in Figure 7.

The Cox proportional hazards model, a regression technique tailored for survival data analysis, was employed to compute hazard ratio (HR) values along with their respective confidence intervals (CIs). Through Cox regression analysis of the 11 lncRNAs under study, it was affirmed that ENSG00000267838, ENSG00000266340, and FRMD6-AS1 are significantly linked to an elevated risk of disease progression in cervical cancer patients. Notably, the lncRNA ENSG00000267838 emerged as particularly significant, marked by a

very low p-value (< 0.001), thereby bolstering its importance as a prognostic biomarker for CC. The lncRNA NORAD, when underexpressed, was associated with a worse prognosis, evidenced by a p-value of 0.051. Conversely, the other lncRNAs did not reach statistical significance, which might indicate a non-systematic association, as depicted in Figure 8.

Sensitivity and specificity analysis

Sensitivity and specificity analysis was conducted to gauge the performance metrics of the model, aimed at delineating its capability to discern varying aspects of treatment response accurately. This method facilitated a deeper exploration of molecular determinants, such as the expression of DEGs, in relation to the efficacy of chemoradiotherapy in CC. The gene ENSG00000267838, as highlighted earlier, showcased a distinct capacity to categorize the 31 patients into responders (R, 21) and non-responders (NR, 10), predicated upon its expression being either positive or negative. This discriminatory power was further corroborated through sensitivity and specificity analysis, leveraging the expression profiles of the 11 DEGs across the 21 R and 10 NR patients. The overall accuracy of the model, considering the full training dataset, was recorded at 87.09%, with the LOOCV accuracy at 77.41%. Sensitivity, or the model's proficiency in correctly identifying true positives, stood at 90.48%, whereas specificity, indicating the accuracy in pinpointing true negatives, was at 80%. These metrics underscored the model's robustness, evidenced by high counts of True Positives (19) and True Negatives (8), alongside lower incidences of False Positives (2) and False Negatives (2), suggesting commendable classification performance. Moreover, the Positive Likelihood Ratio (4.52) and Negative Likelihood Ratio (0.12) further affirm the model's overall efficacy.

Investigation of lncRNAs in cancer

In our thorough exploration of lncRNAs implicated in CC, the AnnoLnc2 tool was instrumental in identifying differentially expressed genes with significant expression in female reproductive tissues and cancers. This approach was designed to encompass the broad spectrum of contexts related to these conditions. Additionally, AnnoLnc2 facilitated the examination of potential interactions between these DEGs and proteins, transcription factors, and miRNAs. The robustness of these interactions was further validated through similarity analyses utilizing BLAST and the UCSC Genome Browser, complemented by a review of scientific literature to confirm the relevance of these genes in CC.

The findings underscore the profound association of these lncRNAs with critical clinical parameters, such as recurrence-free survival, overall survival, tumorigenesis, disease aggressiveness, malignancy, and cellular proliferation. Notably, several lncRNAs were identified as playing key roles in inhibiting apoptosis, enhancing metastasis, contributing to FIGO stage advancement, and facilitating pro-tumorigenic ceRNA networks. For instance, MIR205HG is implicated in CC progression through its function as a pro-tumorigenic ceRNA, interacting with SRSF1, KRT17, miR-16-5p, and FOXP2, thereby presenting a therapeutic avenue for CC diagnosis and treatment [42, 43]. NORAD, linked to a poor prognosis, operates as a ceRNA to foster cell proliferation and invasion by sequestering miR-590-3p [44, 45].

LINC00189 is notable for its predictive value regarding CC recurrence [46, 47], while RUSC1-AS1 serves as a ceRNA regulating BCL-2 protein expression post-transcriptionally [48]. PTPNA-AS1 influences proliferative, apoptotic, and cell cycle dynamics by interacting with miR-876-5p, underscoring its potential as a biomarker [49]. CDKN2B-AS1 plays a role in CC progression through biological process regulation and acts as a ceRNA by binding to miR-181a-5p [50]. MIR210HG promotes CC progression by acting as a ceRNA and regulating the miR-503-5p/TRAF4 axis [51]. The suppression of OIP5-AS1 has been shown to reduce cell viability, invasion, and migration, impacting cell cycle regulation and apoptosis [52, 53].

The identification of lncRNAs ENSG00000267838, ENSG00000266340, and FRMD6-AS1 through decision tree analysis as pivotal in differentiating patient responses highlights their significance in CC treatment stratification. However, the specific mechanisms through which these lncRNAs influence CC remain to be fully elucidated, pointing to the necessity for additional research to unravel their roles and therapeutic potential in CC management.

DISCUSSION

Cervical cancer shows significant global disparities, underscoring the necessity for implementation studies aimed at standardizing care. This includes the integration of advancements such as advanced imaging techniques, radiotherapy, and biological products [73]. The WHO global strategy to eliminate CC articulates targets within the framework of the 90-70-90 strategy: achieving a 90% HPV vaccination rate, 70% CC screening coverage, and 90% treatment provision for women diagnosed with precancerous lesions and invasive

CC [74]. This strategy anticipates a substantial reduction in CC incidence by 2120. Beyond broadening therapy access, a profound comprehension of tumor biology remains paramount [75]. Cutting-edge methodologies like RNA sequencing are instrumental in generating vital genomic insights, facilitating gene expression analysis, biomarker identification, and the elucidation of cancer progression. These insights are crucial for the development of sophisticated therapeutics and diagnostics approaches [76, 77].

The outcomes of the differential expression analysis underscore the identification of 417 lncRNAs with differential expression across the R and NR patient groups. The emergence of unique gene signatures intimates the potential role of these lncRNAs as indicative markers for treatment response in CC. The reversal of gene expression patterns between the R and NR groups underscores the intricate molecular landscape driving the variability in treatment outcomes (Figure 2). Gene ontology analysis revealed that the identified genes, with the notable exception of SNHG29, play roles in CC pathogenesis, including CDKN2B-AS1 [78], NEAT1 [60], MALAT1 [61], and SCARNA9 [62]. These genes are linked to various facets of CC progression, accentuating their utility as molecular targets for both diagnostic and therapeutic applications. The comprehensive evaluation of these results indicates that while specific GO terms may not have achieved statistical significance, the documented involvement of these genes in the scientific literature underscores the critical function of lncRNAs in the context of CC.

The strategic filtering of DEGs culminated in identifying 49 transcripts deemed potentially significant for the biological processes underpinning CC, as delineated in Table 1. The discernible gene signature differentiating the R and NR groups underscores the possibility that these DEGs may influence treatment outcomes or the course of disease progression. This distinction between R and NR groups underscores the biological diversity inherent in CC, accentuating the necessity of tailoring therapeutic strategies to accommodate these variances (Figure 3). The TFs identified (Table 2) play a pivotal role in modulating chromatin structure and gene expression, thereby exerting influence over the tumor microenvironment. Their engagement in the regulation of critical suppressor genes, such as *tp53*, unveils them as potential therapeutic targets, underscoring their significance in immune responses and the anti-tumor mechanisms specific to CC. The proteins analyzed exhibit dual functionality, acting both as tumor promoters and suppressors, particularly influencing apoptosis in response to treatments like paclitaxel, and impacting cell cycle dynamics by keeping cells in the S phase and postponing the onset of mitosis, thus affecting tumor progression and the clinical trajectory of CC.

The DEG-associated miRNAs, implicated in a broad spectrum from overall survival to clinicopathological characteristics, underscore their critical role in modulating cellular processes and the genesis of CC. Their functionality is intricately influenced by their expression profiles, particularly in the context of ceRNA dynamics. Upon refinement, eight DEGs of relevance were pinpointed (Figure 4). The selected lncRNAs for this refined analysis, including MIR205HG, NORAD, LINC00189, RUSC1-AS1, PITPNA-AS1, CDKN2B-AS1, MIR210HG, and OIP5-AS1, had their associations with CC substantiated through scientific literature and validated by resources such as the UCSC Genome Browser, solidifying their potential roles and the need for further investigation in the context of CC.

The decision tree analysis elucidated the connection between the expression profiles of selected differentially expressed genes, namely ENSG00000267838, ENSG00000266340, and FRMD6-AS1, and the response to treatment. Particularly, ENSG00000267838 demonstrated differential expression in female reproductive tissues, with a marked decrease in expression observed in CC, underscoring its significance within this context. Its interaction with microRNAs, for example, miR-10-5p, implies an impact on cell viability, emphasizing its importance in CC [39]. Further decision tree analysis focusing exclusively on ENSG00000267838 assessed this lncRNA's expression in R and NR groups. This detailed examination confirmed ENSG00000267838 potential as a discriminative marker between R and NR groups, suggesting that its downregulation could be indicative of a positive response to treatment, whereas upregulation might mean treatment resistance.

Kaplan-Meier survival analysis indicated that lower expression levels of NORAD are associated with worse outcomes, suggesting that lncRNAs as a pivotal factor in CC progression and potential therapeutic target. Conversely, upregulation of ENSG00000267838, FRMD6-AS1, and ENSG00000266340 was associated with increased disease progression risk, highlighting their significance in patient prognosis. Cox model survival analysis further underscored the critical role of lncRNAs ENSG00000267838, ENSG00000266340, and FRMD6-AS1, associating them with a significant elevation in progression risk. The statistical robustness of these findings, demonstrated through low p-values, affirms the strong link between the overexpression of these genes and adverse outcomes in CC patients, advocating for their consideration as markers for prognosis and potential therapeutic intervention.

The investigation into the role of lncRNAs in CC underscores the significance of their expression levels in evaluating disease progression risk. The lncRNAs PITPNA-AS1, NORAD, and MIR205HG exhibited statistically significant correlations with CC prognosis, with their under-expression linked to a worse prognosis. This association accentuates the

importance of these lncRNAs as potential markers for disease progression. Although LINC00189 suggested a positive prognosis when overexpressed, the lack of statistical significance calls for further research to confirm its role.

The positive and negative likelihood ratios further attest to the model's overall efficiency, offering a foundation for discussions on its robustness and potential clinical utility. MIR205HG stands out as a multifunctional competing endogenous RNA (ceRNA), affecting the expression of genes involved in key cellular processes such as proliferation, apoptosis, and migration. Other lncRNAs like NORAD, LINC00189, RUSC1-AS1, PITPNA-AS1, CDKN2B-AS1, MIR210HG, and OIP5-AS1 have shown significant contributions to various facets of CC progression, positioning them as critical regulators and potential therapeutic targets. The identification of ENSG00000267838, ENSG00000266340, and FRMD6-AS1 as pivotal in distinguishing between patient groups via the decision tree analysis underscores the necessity for further research to elucidate their specific roles in influencing chemoradiotherapy responses.

The performance metrics of the model, with a sensitivity of 90.48% and specificity of 80%, demonstrate its effectiveness in correctly classifying patients into responder and non-responder groups. The high rates of true positives and true negatives, coupled with low rates of false positives and false negatives, highlight the model's reliability.

A deeper understanding of their mechanisms is crucial for developing more effective therapeutic strategies. Thus, additional studies are required to validate these findings and to enhance our comprehension of the dynamics and influence of these DEGs in CC, aiming to establish a more precise signature for treatment response.

CONCLUSION

In summary, despite ongoing efforts to screen for CC and investigate biomarkers, there remains a pressing need for further research and the exploration of new avenues. This study underscores the pivotal role of lncRNAs in influencing therapeutic responses via their expression profiles, thereby reinforcing their potential utility as biomarkers for the diagnosis, prognosis, and monitoring of CC. Our findings particularly spotlight three lncRNAs—ENSG00000267838, FRMD6-AS1, and ENSG00000266340—as crucial in differentiating patient responses to therapy based on their regulatory profiles. The under-expression of MIR205HG, NORAD, and PITPNA-AS1 was linked to a less favorable prognosis, whereas the over-expression of ENSG00000267838, FRMD6-AS1, and ENSG00000266340 was

indicative of a higher risk of disease progression. Among these, lncRNA ENSG00000267838 emerges as a standout biomarker, exhibiting a significant capability to segregate responders from non-responders, highlighting its potential in guiding therapeutic decisions and patient management in CC.

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List of Figures

Fig. 1 – Pipeline describing the steps to identify and characterize lncRNAs associated with response to chemoradiotherapy treatment for CC. The steps are divided into the following

categories: obtaining data from patient samples; sample preparation; identification of DEGs; refinement of DEGs; statistical analysis; and investigation of the role of these lncRNAs in cancer.

Fig. 2 – Heatmap representing 417 DEGs comparing patients 21 R to 10 NR. LogFoldChange > 1 or < -1 and padj value < 0.05 were considered. Indicators: "Parametrial involvement" represents parametrial involvement as free (salmon), unilateral (brown) or bilateral (aqua green). "Histologic grade" is histologic grade II (light pink) or III (dark pink). "FIGO stage" shows which FIGO grade the patient is in, IIA (gold), IIB (dark green) or IIIB (light purple). "Final result" is the treatment status, R (dark purple) or NR (orange).

Fig. 3 – Heatmap representing 49 DEGs associated with CC. Comparison between 21 R and 10 NR patients. LogFoldChange > 1 or < -1 and padj value < 0.05 were considered. Positively regulated DEGs are shown in red, negatively regulated DEGs in blue and those with constant expression in white. Light red indicates DEGs with a z score < 1 , while light blue indicates DEGs with a z score > -1 . The intense red and blue colors represent DEGs with a z score > 1 or < -1 .

Fig. 4 – Schematic representing the DEG refinement process, which resulted in the selection of 8 DEGs.

Fig. 5 – Decision tree generated for the 49 DEGs according to the gene expression profile for the 31 patients. Using the full data set (Full training) to classify the records, the algorithm found 28/31 (90.32%) possibilities. While the LOOCV validation method correctly classified 25/31 hits, i.e. 80.64% accuracy.

Fig. 6 – Decision tree generated for lncRNA ENSG00000267838. The LOOCV accuracy was 80.64, with a value of 87.09% and the FULL training value was 87.09%.

Fig. 7 – Demonstrating a satisfactory separation between the R and NR groups. Kaplan-Meier graphs for the 11 genes that stood out as possible biomarkers. **a)** RUSC1-AS1 showed a relationship with a worse prognosis when negatively regulated, but with no significant difference. **b)** OIP5-AS1 also showed no significant difference, and its profile does not make it possible to say that it is a classic profile of underexpression and overexpression. **c)**

MIR205HG shows a direct impact on the progression of CC, being associated with a worse prognosis when negatively regulated, and its positive regulation shows a protective effect, with a significant difference. **d)** MIR210HG showed a high risk when negatively regulated and a protective effect when positively regulated, but with no significant difference. **e)** NORAD showed high risk when negatively regulated and a protective effect under positive regulation, with a significant difference. **f)** PITPNA-AS1 has a risk effect under negative regulation and a protective effect under positive regulation, with a significant difference. **g)** CDKN2B-AS1 showed high risk when negatively and positively regulated, with no significant difference. **h)** LINC00189 proved to be associated with a worse prognosis when negatively and positively regulated, with no significant difference. **i)** ENSG00000267838 showed a high risk when positively regulated, with a significant difference. **j)** FRMD6-AS1 showed a high risk when positively regulated, with a significant difference. **k)** ENSG00000266340 showed a high risk when positively regulated, with a significant difference.

Fig. 8 – Survival analysis. Evaluation with Hazard Ratio values to assess transcripts with the potential to be used as biomarkers.

Table 1 – Table containing the ENSEMBL ID, the symbol and the description of the 49 DEGs selected for their involvement with tissues and cancers of the female reproductive system.

ENSEMBL ID	SYMBOL	DESCRIPTION
ENSG00000215533	<i>LINC00189</i>	Long Intergenic Non-Protein Coding RNA 189
ENSG00000225855	<i>RUSC1-AS1</i>	RUSC1 Antisense RNA 1
ENSG00000230937	<i>MIR205HG</i>	MIR205 host gene
ENSG00000236618	<i>PITPNA-AS1</i>	PITPNA Antisense RNA 1
ENSG00000238142	LOC105376805	Uncharacterized LOC105376805
ENSG00000240498	<i>CDKN2B-AS1</i>	CDKN2B Antisense RNA 1
ENSG00000244151	RP11-148K1.12	Novel transcript
ENSG00000247095	<i>MIR210HG</i>	MIR210 Host Gene
ENSG00000247556	<i>OIP5-AS1</i>	OIP5 Antisense RNA 1
ENSG00000248980	SPCS3-AS1	SPCS3 Antisense RNA 1
ENSG00000251562	MALAT1	Metastasis Associated Lung Adenocarcinoma Transcript 1
ENSG00000257379	RP11-793H13.8	Novel transcript

ENSG00000257553	RP11-603J24.17	Novel transcript
ENSG00000257663	RP11-1100L3.7	Novel transcript
ENSG00000258017	RP11-386G11.10	Novel transcript
ENSG00000258232	RP11-161H23.5	Novel transcript
ENSG00000259865	RP11-488L18.10	Novel transcript
ENSG00000259972	AC009120.6	Novel transcript
ENSG00000260032	<i>NORAD</i>	Non-Coding RNA Activated By DNA Damage
ENSG00000261061	RP11-303E16.2	Novel transcript
ENSG00000261183	SPINT1-AS1	SPINT1 Antisense RNA 1
ENSG00000264577	AC010761.8	Novel transcript
ENSG00000265401	RP11-138I1.4	Novel transcript
ENSG00000266340	RP11-848P1.4	Novel transcript
ENSG00000266402	SNHG25	Small Nucleolar RNA Host Gene 25
ENSG00000267165	CHMP1B-AS1	CHMP1B Antisense RNA 1
ENSG00000267523	CTD-2537I9.12	Novel transcript
ENSG00000267815	CTB-191K22.5	Novel transcript
ENSG00000267838	AC008746.12	Novel transcript
ENSG00000269243	CTD-2231E14.8	Novel transcript
ENSG00000269680	CTD-3128G10.6	Novel transcript
ENSG00000269893	SNHG8	Small Nucleolar RNA Host Gene 8
ENSG00000269958	RP11-73M18.8	Novel transcript
ENSG00000272182	RP11-802O23.3	Novel transcript
ENSG00000272234	CTD-2325A15.5	Novel transcript
ENSG00000272288	ILRUN-AS1	Novel transcript
ENSG00000272696	RP11-339B21.13	Novel transcript
ENSG00000272933	TRIM8-DT	TRIM8 Divergent Transcript
ENSG00000273149	RP11-290D2.6	Novel transcript
ENSG00000273449	RP11-218F10.3	Novel transcript
ENSG00000273888	<i>FRMD6-AS1</i>	FRMD6 Antisense RNA 1
ENSG00000276570	CTD-2587H24.14	Novel transcript
ENSG00000277801	RP11-681H18.2	Novel transcript
ENSG00000277978	RP11-403P17.6	Novel transcript
ENSG00000279364	RP11-463I20.1	TEC (To be Experimentally Confirmed)
ENSG00000279605	-	TEC (To be Experimentally Confirmed)
ENSG00000279753	-	TEC (To be Experimentally Confirmed)
ENSG00000280027	-	Novel transcript
ENSG00000280064	-	Novel transcript

Table 2 – Table containing the symbol of the transcription factors, their name, their function in cervical cancer and their interactions with the 49 selected lncRNAs, located by AnnoLnc2.

TFs	Name	Function in the CC	LncRNAs of interaction
AMH	Anti-Müllerian Hormone	Tumor suppressor. [1]	LINC00189, RUSC1-AS1, ENSG00000238142, CDKN2B-AS1, MIR210HG, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258232, ENSG00000265401, SNHG25, ENSG00000272182, ENSG00000272234, ENSG00000273449, ENSG00000277801
ASF1A	Anti-Silencing Function 1A Histone Chaperone	Correlation with increased rates of cancer progression and occurrence of metastases. Contributes to DNA repair and cellular senescence. [2]	LINC00189, RUSC1-AS1, CDKN2B-AS1, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258232, CHMP1B-AS1, SNHG8, TRIM8-DT
BRCA1	Breast Cancer 1	Expression related to survival, low expression is associated with a worse prognosis. [3]	LINC00189, PITPNA-AS1, CDKN2B-AS1, ENSG00000244151, OIP5-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258232, ENSG00000273888, NORAD, ENSG00000265401, SNHG25, CHMP1B-AS1, SNHG8, ILRUN-AS1, TRIM8-DT, ENSG00000273149, ENSG00000279605
BRD4	Bromodomain Containing 4	Repression leads to significant radiosensitization and potentiation of DNA damage. [4]	LINC00189, RUSC1-AS1, ENSG00000238142, ENSG00000244151, MIR210HG, OIP5-AS1, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258232, NORAD, NORAD, ENSG00000265401, CHMP1B-AS1, SNHG8, TRIM8-DT, ENSG00000273149, ENSG00000279605

CEBPB (C/EBP β)	CCAAT Enhancer Binding Protein Beta	Overexpression can inhibit cell proliferation, migration, invasion, arrest cells in S phase and promote apoptosis. [5]	LINC00189, RUSC1-AS1, PITPNA-AS1, CDKN2B-AS1, ENSG00000244151, OIP5-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258017, ENSG00000258232, SPINT1-AS1, ENSG00000264577, SNHG25, CHMP1B-AS1, ENSG00000267523, ENSG00000267815, ENSG00000269243, ENSG00000269680, SNHG8, ILRUN-AS1, TRIM8-DT, ENSG00000273149, ENSG00000279364, ENSG00000279605
CTCF	CCCTC-Binding Factor	Integrated into HPV, it reorganizes the chromatin state and positively regulates genes essential for tumor viability. [6]	LINC00189, RUSC1-AS1, PITPNA-AS1, ENSG00000238142, CDKN2B-AS1, ENSG00000244151, MIR210HG, OIP5-AS1, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258232, ENSG00000273888, ENSG00000259972, NORAD, ENSG00000265401, SNHG25, CHMP1B-AS1, ENSG00000267523, ENSG00000267815, ENSG00000267838, ENSG00000269243, SNHG8, ENSG00000272234, TRIM8-DT, ENSG00000273149, ENSG00000279364, ENSG00000279605, ENSG00000279753
DEK	DEK proto-oncogene	Overexpression inhibits apoptosis and senescence in HeLa cells, and prevents tp53 transcriptional activity. [7]	LINC00189, RUSC1-AS1, PITPNA-AS1, CDKN2B-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000258232, ENSG00000259972, NORAD, ENSG00000265401, SNHG25, CHMP1B-AS1, ENSG00000267523, TRIM8-DT, ENSG00000279605.
E2F1	E2F transcription factor 1	Prognostic factor, favorable when overexpressed. Up-regulation promotes apoptosis through tp53-dependent and -independent pathways. [8]	RUSC1-AS1, PITPNA-AS1, ENSG00000238142, CDKN2B-AS1, ENSG00000244151, MIR210HG, OIP5-AS1, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258017, ENSG00000258232, ENSG00000273888, NORAD, ENSG00000265401, SNHG25, CHMP1B-AS1, ENSG00000267523,

			SNHG8, ILRUN-AS1, ENSG00000272696, TRIM8-DT, ENSG00000273149, ENSG00000279605
ELK1	ETS Transcription Factor ELK1	Increased expression represses apoptosis and autophagy in CC cells. [9] [10]	LINC00189, PITPNA-AS1, CDKN2B-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000258232, NORAD, ENSG00000265401, ENSG00000273149
ELK4	ETS Transcription Factor ELK4	Promotes cell cycle progression. [11]	LINC00189, RUSC1-AS1, PITPNA-AS1, CDKN2B-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000258017, ENSG00000258232, NORAD, ENSG00000265401, SNHG25, CHMP1B-AS1, ENSG00000272234, TRIM8-DT, ENSG00000273149
EP300	E1A Binding Protein P300	Inhibitor of the anti-tumor immune response in CC via metabolic modulation. [12] [13]	LINC00189, ENSG00000264577, RUSC1-AS1, PITPNA-AS1, CDKN2B-AS1, ENSG00000244151, OIP5-AS1, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258232, ENSG00000273888, ENSG00000259972, NORAD, ENSG00000265401, CHMP1B-AS1, SNHG8, ILRUN-AS1, TRIM8-DT, ENSG00000273149
FOXM1	Forkhead Box M1	Drug resistance and poor prognosis. [14] [15]	LINC00189, RUSC1-AS1, PITPNA-AS1, ENSG00000244151, OIP5-AS1, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258017, ENSG00000258232, NORAD, SNHG25, CHMP1B-AS1, SNHG8, ENSG00000273149
JUND	JunD proto-oncogene	Increases cell proliferation and the ability of cancer cell lines to invade. [16]	LINC00189, RUSC1-AS1, CDKN2B-AS1, OIP5-AS1, MALAT1, ENSG00000257379, ENSG00000258232, ENSG00000259972, NORAD, SPINT1-AS1, SNHG25, CHMP1B-AS1

MCM7	Minichromosome Maintenance Complex Component 7	Associated with high-grade cervical lesions, tumor formation, progression and malignant conversion. [17] [18] [19]	LINC00189, RUSC1-AS1, ENSG00000238142, ENSG00000244151, MIR210HG, OIP5-AS1, SPCS3-AS1, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258232, NORAD, ENSG00000265401, SNHG25, ENSG00000267523, SNHG8, ENSG00000272234, ENSG00000279605, ENSG00000279753
MYBL2	MYB Proto-Oncogene Like 2	Expression levels affected by HPV 16. Expression levels are related to pathological changes in the cervix and may induce the development of CC. [20] [21] [22]	LINC00189, CDKN2B-AS1, MALAT1
MYC	MYC proto-oncogene	Simultaneous overexpression of HPV amplification, which is associated with the acquisition of a malignant phenotype in cervical cells. It is an oncogene involved in tumor progression. [23] [24]	LINC00189, RUSC1-AS1, PITPNA-AS1, CDKN2B-AS1, ENSG00000244151, MIR210HG, OIP5-AS1, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258017, ENSG00000258232, ENSG00000273888, NORAD, SPINT1-AS1, ENSG00000265401, SNHG25, CHMP1B-AS1, ENSG00000269243, SNHG8, ILRUN-AS1, TRIM8-DT, ENSG00000273149, ENSG00000279605
NFE2L2	NFE2 Like BZIP Transcription Factor 2	It is associated with a poor prognosis and can affect the tumor microenvironment and macrophage infiltration. [25]	LINC00189, RUSC1-AS1, PITPNA-AS1, CDKN2B-AS1, OIP5-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000258232, NORAD, SNHG25, CHMP1B-AS1, SNHG8, TRIM8-DT, ENSG00000273149
NR2C2	Nuclear receptor subfamily 2, group C, member 2	Related to apoptosis mediated by DNA damage induced by ionizing radiation. [26]	LINC00189, RUSC1-AS1, PITPNA-AS1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258232, NORAD
ORC1	Origin Recognition Complex subunit 1	Overexpression accelerates the progression of CC, promoting proliferation and reducing apoptosis.	RUSC1-AS1, MIR210HG, SPCS3-AS1, MALAT1, ENSG00000257553, ENSG00000257663, ENSG00000273888, NORAD, CHMP1B-AS1, ENSG00000267523

[\[27\]](#)

PTEN	Phosphatase and Tensin Homolog	Under-expression is involved in pathogenesis, invasion and metastasis. [28]	LINC00189, MIR205HG, PITPNA-AS1, ENSG00000238142, CDKN2B-AS1, ENSG00000244151, MIR210HG, OIP5-AS1, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000259972, NORAD, SPINT1-AS1, ENSG00000265401, CHMP1B-AS1, ENSG00000269243, TRIM8-DT
PYGO2	Pygopus Family PHD Finger 2	Essential for the growth of CC cell lines. [29]	LINC00189, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, NORAD, SNHG25
RAD21	RAD21 Cohesin Complex Component	Associated with survival and susceptibility to CC. [30]	LINC00189, RUSC1-AS1, PITPNA-AS1, CDKN2B-AS1, ENSG00000244151, MIR210HG, OIP5-AS1, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258232, ENSG00000273888, ENSG00000259972, NORAD, SPINT1-AS1, ENSG00000265401, SNHG25, CHMP1B-AS1, ENSG00000267523, ENSG00000267815, ENSG00000267838, ENSG00000269243, SNHG8, ILRUN-AS1, TRIM8-DT, ENSG00000273149, ENSG00000279364, ENSG00000279605
SFMBT1	Scm like with Four MBT domains 1	Overexpression induced EMT and increased migration and invasion. [31]	LINC00189, RUSC1-AS1, MALAT1, SNHG25, ENSG00000280064

STAT1	Signal Transducer and Activator of Transcription 1	Associated with the progression of cervical lesions. Its expression can be affected by the viral load of HPV16. Increases the sensitivity of cervical tumor cells to chemotherapy drugs and radiation. [32] [33]	LINC00189, RUSC1-AS1, PITPNA-AS1, CDKN2B-AS1, ENSG00000244151, MIR210HG, OIP5-AS1, SPCS3-AS1, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258232, ENSG00000259972, NORAD, SPINT1-AS1, SNHG25, CHMP1B-AS1, SNHG8, ILRUN-AS1, ENSG00000273149
STAT3	Signal Transducer and Activator of Transcription 3	Promotes proliferation and migration skills. [34]	LINC00189, CDKN2B-AS1, MALAT1, ENSG00000257379
TFAP2A	Transcription Factor AP-2 Alpha	Associated with a higher tumor stage, lymph node metastasis and poor survival. [35]	LINC00189, RUSC1-AS1, CDKN2B-AS1, MIR210HG, MALAT1, ENSG00000257379, ENSG00000257553, ENSG00000257663, ENSG00000258232, ENSG00000273888
YY1	YY1 transcription factor	It acts on the progression of HPV-positive CC by regulating the expression of viral oncogenes E6 and E7. It is involved in the tumorigenesis process. When inhibited, it induces tp53 activation and apoptosis in HPV-infected HeLa cells. [36]	RUSC1-AS1, PITPNA-AS1, ENSG00000244151, OIP5-AS1, MALAT1, ENSG00000257379, ENSG00000258017, ENSG00000258232, NORAD, SNHG25, SNHG8, ENSG00000273149

Table 3 – Table containing the symbol of the proteins, their name, their action in the CC and the interactions with the 49 selected lncRNAs localized by AnnoLnc2.

Protein	Name	Function in the CC	LncRNAs of interaction
AEG-1/ MTDH	Astrocyte Elevated Gene-1/ Metadherin	Involved in EMT control. Induces chemoresistance. Up-regulation is correlated with tumor growth and lymph node metastasis. AEG-1 knockdown weakens the stem-like property of HeLa cells. AEG-1 staining is related to the increasing degree of the lesion in cervical intraepithelial neoplasia (CIN). [37] [38] [39]	SNHG8

ALKBH5	AlkB Homolog 5, RNA Demethylase	High expression is associated with a poor prognosis in patients and the induction of tumorigenesis and metastasis. [40]	ENSG00000279605
ARHGEF28	Rho guanine nucleotide exchange factor 28	It is prognostic for CC, high expression is favorable. Mutations influence the survival rate. [41]	ENSG00000258017
ASPH	Aspartate Beta-Hydroxylase	High expression is not favorable for the prognosis of CC. Studies show high expression in patients resistant to radiotherapy, as well as lower overall survival and progression-free survival. [42] [43]	ENSG00000279605
ATXN2	Ataxin 2	Acts as a direct RNA-binding protein (RBP) in CC HeLa cells. It is involved in the regulation of global RNA processing and ribosomal translation. [44]	ILRUN-AS1, ENSG00000277978, SNHG8, ENSG00000257663, RUSC1-AS1, ENSG00000266340, ENSG00000259972, ENSG00000259865, MALAT1, ENSG00000269680, MIR205HG, FRMD6- AS1, OIP5-AS1, ENSG00000276570, NORAD, ENSG00000279605
CAPRN1	Cycle Associated Protein 1	Involved in cell proliferation in Hela cells from CC. [45]	ENSG00000279605, NORAD, SNHG8, ENSG00000269680, ENSG00000276570, ENSG00000269958
CCAR1	Cell Division Cycle And Apoptosis Regulator 1	Expressed at all stages, suggesting a role in driving cell cycle progression. Acts as a coactivator of the tumor suppressor protein tp53. [46]	SPINT1-AS1, ENSG00000280064, ENSG00000266340, MIR205HG, MALAT1, OIP5-AS1, FRMD6- AS1, ENSG00000279364, ENSG00000269958, NORAD, ENSG00000279605, ENSG00000261061

CIRBP	Cold Inducible RNA Binding Protein	It is prognostic of CC, high expression is favorable. Involved in cell metabolism and DNA methylation as prognostic molecular biomarkers in gynecological cancer. [47] [48]	ENSG00000279605, NORAD
DDX3X	DEAD-box helicase 3 X-linked	It plays an oncogenic role in CC. [49] [50]	ILRUN-AS1, SNHG8, ENSG00000279605, ENSG00000238142
DGCR8	DGCR8 Microprocessor Complex Subunit	Overexpressed in CC. Its expression is increased by HPV16 E7 to help accelerate cell proliferation and inhibit paclitaxel-induced cell apoptosis. [51] [52]	ENSG00000279605, NORAD, TRIM8-DT
DROSHA	Drosha ribonuclease III	Increased expression in HPV-positive lineages by the viral oncoproteins E6 and E7 leads to miRNA alterations associated with greater cell motility in CC. [53] [54]	ENSG00000261061, ENSG00000267838, ENSG00000279753, MIR210HG, ENSG00000272696, ENSG00000259865, LINC00189, MIR205HG, OIP5-AS1, FRMD6-AS1, ENSG00000279364, ENSG00000276570, ENSG00000269958, ENSG00000279605, PITPNA-AS1
EIF3A	Eukaryotic Translation Initiation Factor 3 Subunit A	High expression is associated with better survival and a better prognosis, as its expression is completely lost in CC after the cells reach a differentiated status. [55]	ENSG00000264577, ENSG00000257663, ENSG00000258017, MALAT1, ENSG00000269680, ENSG00000279605, ENSG00000273149
EIF3D	Eukaryotic Translation Initiation Factor 3 Subunit D	Promotes tumor progression. [56]	ENSG00000273149, ENSG00000279364, ENSG00000279605, NORAD
ELAVL1	ELAV like RNA binding protein 1	Promotes the growth of cancer cells by regulating RNA in the cell cytoplasm. [57]	SNHG8

EP300	E1A Binding Protein P300	Decreased expression in CC, reducing histone lactation modification and transcriptional mutation of cervical cancer cells, destabilizing cancer cell proliferation. [58]	ENSG00000264577, ENSG00000257663, ENSG00000259972, ENSG00000259865, MALAT1, NORAD, FRMD6-AS1, ENSG00000269680, ENSG00000269958, ENSG00000279605
EPPK1	Epiplakin 1	Expression positively correlated with tumor size in clinicopathological characteristics. [59]	SNHG25, ENSG00000257663, ENSG00000258017, ENSG00000259972, MALAT1, ENSG00000269958, ENSG00000279605
HELLS	Helicase, Lymphoid Specific	HELLS is prognostic, high expression is favorable in CC. Promotes CC proliferation by regulating Nrf2-mediated ferroptosis. [60] [61]	ENSG00000257553, ENSG00000273449, ENSG00000276570
HNRNPU	heterogeneous nuclear ribonucleoprotein U	It has CC proliferative properties. [62]	MIR205HG, NORAD, ENSG00000269958, ENSG00000279605
HUWE1	HECT, UBA And WWE Domain Containing E3 Ubiquitin Protein Ligase 1	Negative regulation contributes to the proliferation of CC. [63]	ENSG00000277801, NORAD, MIR210HG, ENSG00000266340, ENSG00000258017, ENSG00000259865, MIR205HG, OIP5-AS1, FRMD6-AS1, ENSG00000279364, ENSG00000269958, ENSG00000279605
LRP11	LDL Receptor Related Protein 11	It acts on proliferation, migration and invasion. It is highly expressed in high-grade squamous intraepithelial lesions and is correlated with the overall survival rate. [64]	ENSG00000276570
NUDT21	Nudix Hydrolase 21	It is a tumor suppressor to inhibit the progression of CC. [65]	ENSG00000277978, SNHG8

PRKDC	Protein Kinase, DNA-Activated, Catalytic Subunit	Associated with a high mutation load in CC. [66]	ENSG00000257553, ENSG00000273449, MIR210HG, ENSG00000244151, ENSG00000258017, LINC00189, OIP5-AS1, ENSG00000269680, FRMD6-AS1, ENSG00000279364
VIRMA	Vir Like M6A Methyltransferase Associated	It has tumor-promoting effects. [67]	PITPNA-AS1, ENSG00000267523, ENSG00000273449, ENSG00000279753, MIR210HG, ENSG00000272696, LINC00189, MIR205HG, OIP5-AS1, ENSG00000279364
YTHDF2	YTH N6- methyladenosine RNA binding protein F2	Expression is up-regulated in CC tissues. Low expression can restrict proliferation, promote apoptosis and arrest cells in S phase in cervical cancer cells. [68]	ILRUN-AS1, SNHG8, ENSG00000279605

Table 4 – Table containing the identification of miRNAs, their function in the CC and their interactions with the 49 selected lncRNAs that were localized by AnnoLnc2.

miRNAs	Function in the CC	LncRNAs of interaction
miR-10-5p	Inhibition of cancer cell viability. Induction of cell cycle arrest. [69] [70]	PITPNA-AS1, CDKN2B-AS1, ENSG00000244151, MIR210HG, ENSG00000257379, ENSG00000258017, ENSG00000259972, ENSG00000265401, ENSG00000266340, ENSG00000267838, ENSG00000269243, ENSG00000272234, TRIM8-DT, ENSG00000276570, ENSG00000279605.
miR-122-5p	Modulation of radiosensitivity. [71] [72]	LINC00189, MIR205HG, CDKN2B-AS1, NORAD, ENSG00000261061, ENSG00000266340, ENSG00000267815, ENSG00000269243, ILRUN- AS1, ENSG00000272696, ENSG00000276570, ENSG00000277978, ENSG00000280027.
miR-124-3p	Suppresses proliferation, migration and invasion of CC cells. [73]	LINC00189, CDKN2B-AS1, MALAT1, ENSG00000265401, ENSG00000266340, ENSG00000267838, ENSG00000269243, ILRUN- AS1, ENSG00000272696, TRIM8-DT, ENSG00000280064.

miR-128-3p	Inhibition of proliferation, migration and invasion of CC cells. [74] [75]	ENSG00000244151, SPCS3-AS1, ENSG00000257663, CHMP1B-AS1, ENSG00000267838, ENSG00000269958, ENSG00000272696, ENSG00000273149, ENSG00000277801.
miR-129-5p	Inhibition of the effects of CC cell migration and invasion. [76]	OIP5-AS1, PITPNA-AS1, ENSG00000258232, ENSG00000259972, ENSG00000261061, ENSG00000264577, CHMP1B-AS1, ENSG00000267523, ENSG00000272234, TRIM8-DT, ENSG00000273149, ENSG00000273449, ENSG00000279364, ENSG00000279753, ENSG00000280027.
miR-140-3p	Prevents the proliferation of human CC cells. [77] [78]	ENSG00000257553, ENSG00000258017, ENSG00000258232, SPINT1-AS1, ENSG00000277801.
miR-140-5p	The level of expression increases/decreases cell proliferation, migration and invasion in CC cells. [79] [80]	ENSG00000257553, ENSG00000266340, ENSG00000269680.
miR-143-3p	Suppresses the occurrence and development of CC. [81]	ENSG00000277801, ENSG00000279753.
miR-145-5p	Regulates proliferation, migration and invasion of CC cells. [82]	PITPNA-AS1.
miR-150-5p	Promotes proliferation and EMT of CC cells. [83]	ENSG00000257553, ENSG00000257663, ENSG00000272234.
miR-182-5p	Tumor suppressor reported in CC. [84]	SPINT1-AS1.
miR-214-5p	Involved in radioresistance in the CC. [85]	ENSG00000277978.
miR-375	Positive regulation influences acquired resistance to paclitaxel in CC. Negative regulation involved in pelvic lymph node metastasis and FIGO stage. [86] [87] [88] [89]	LINC00189, SPCS3-AS1, ENSG00000257553, ENSG00000258232, SPINT1-AS1, CHMP1B-AS1, ILRUN-AS1, ENSG00000272696, ENSG00000273149, ENSG00000273449, FRMD6-AS1, OIP5-AS1.

Figure 1

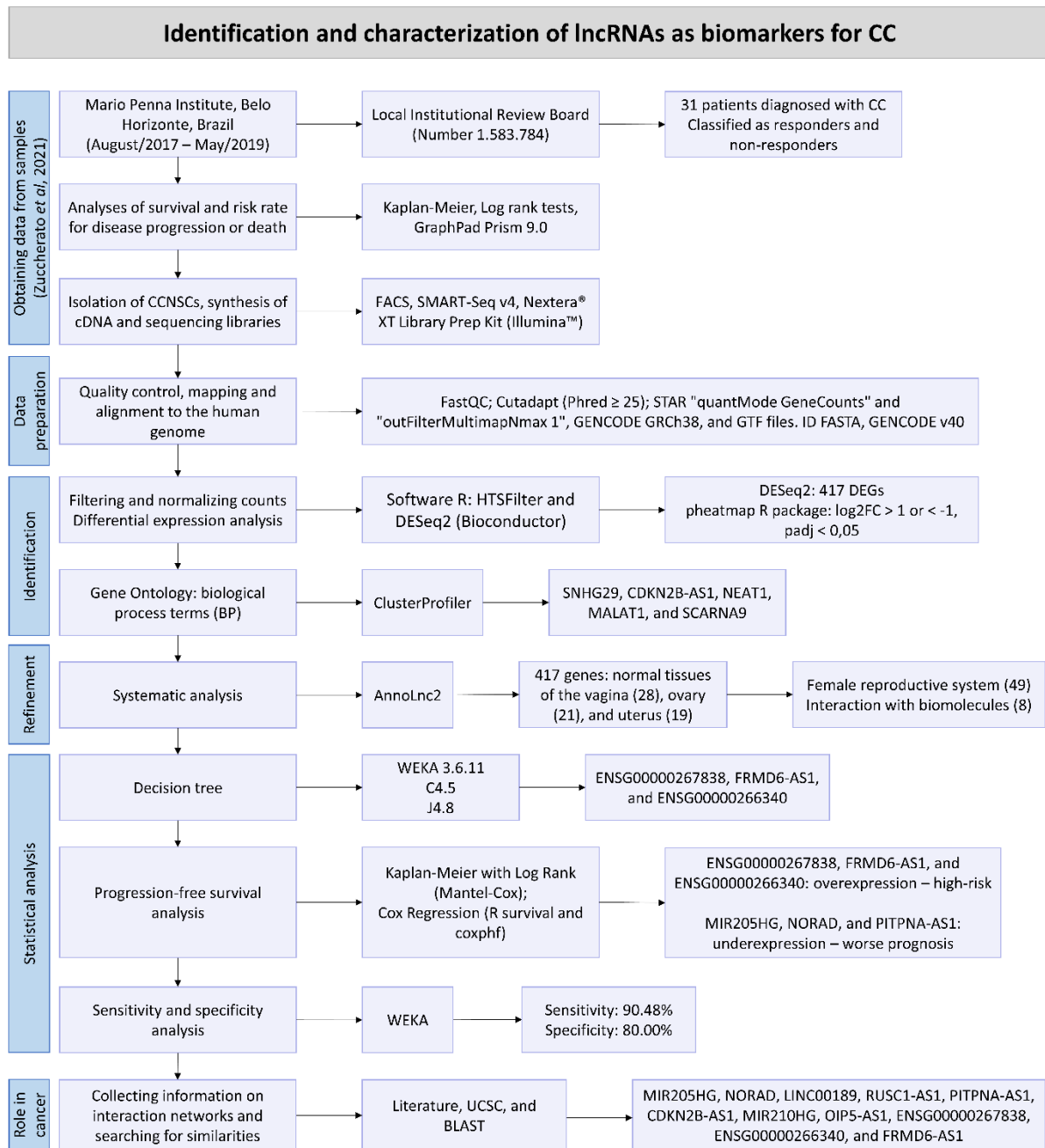


Figure 2

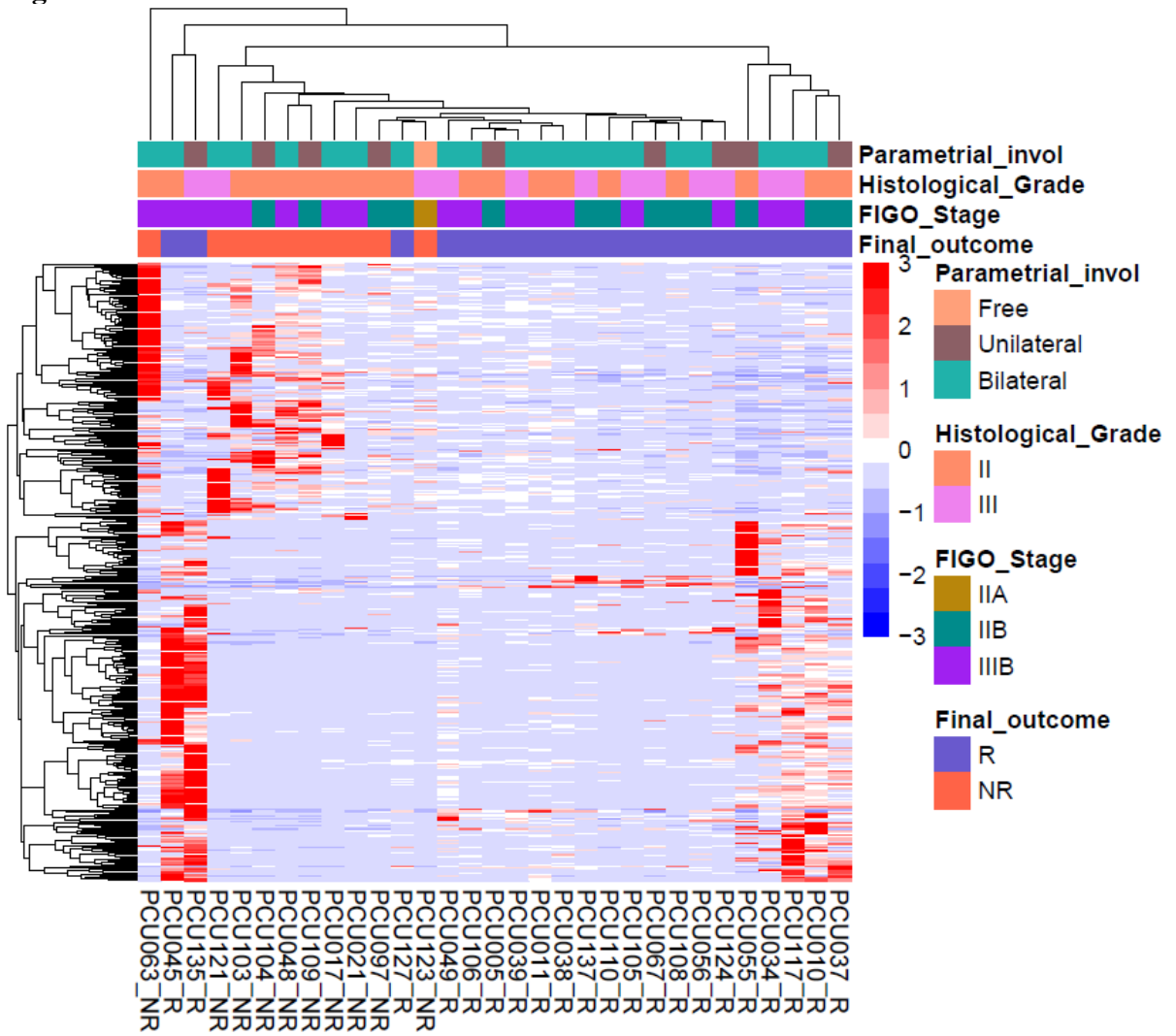


Figure 3

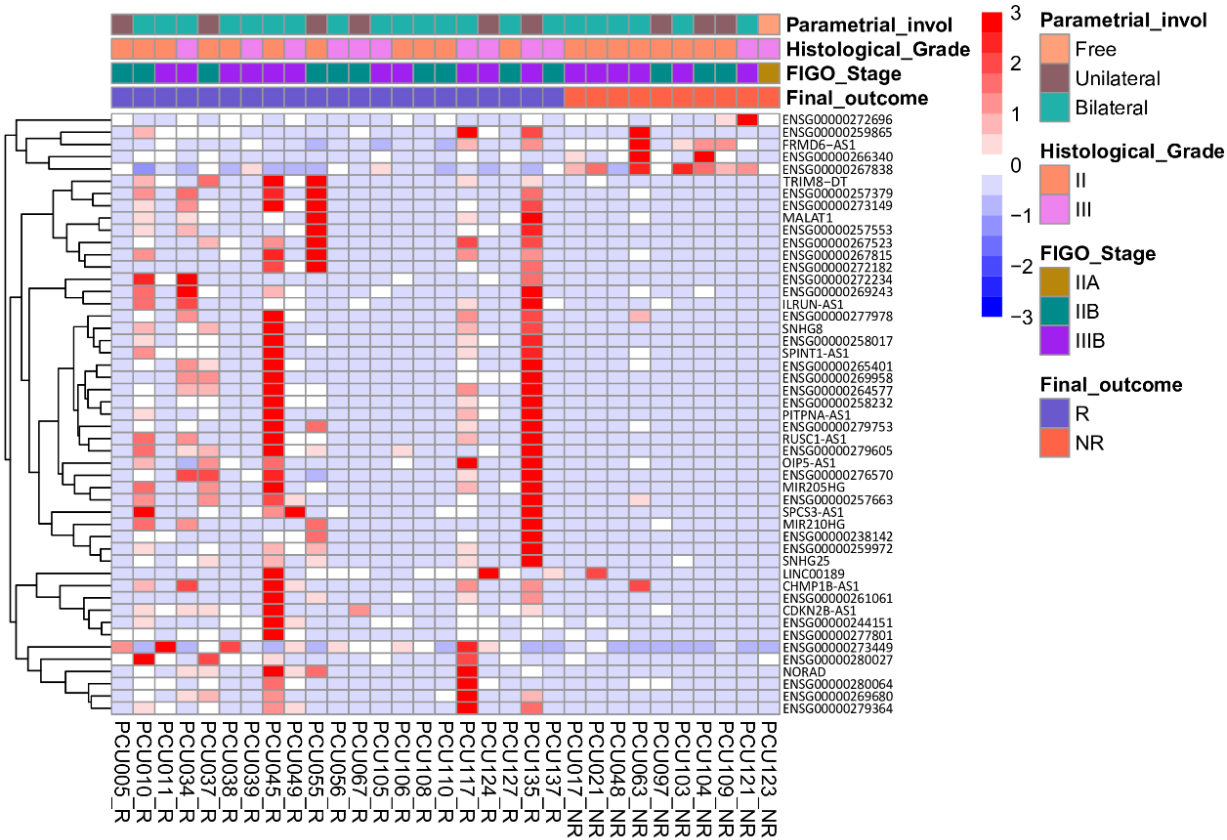


Figure 4

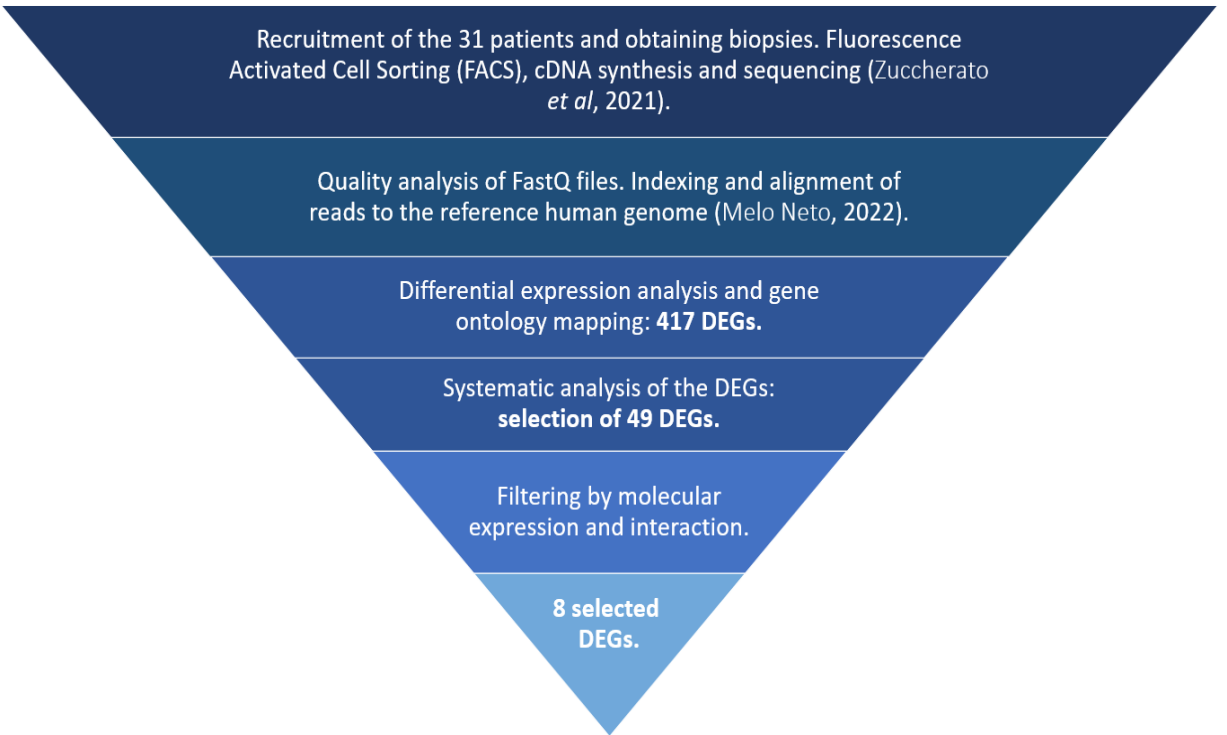


Figure 5

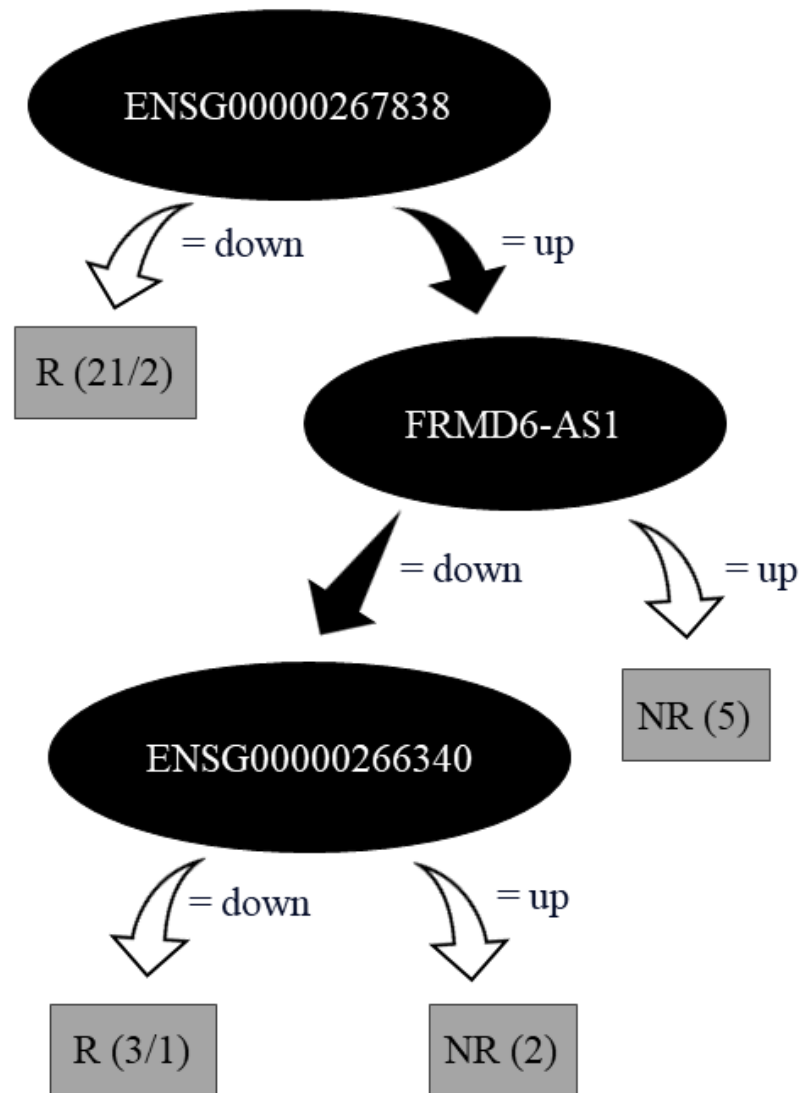


Figure 6

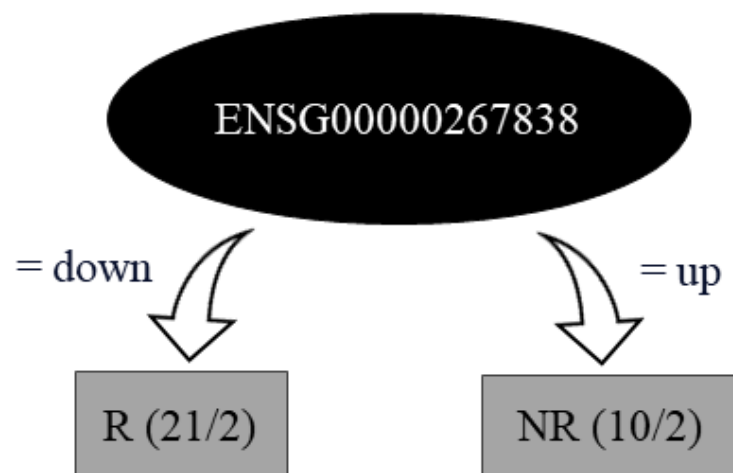
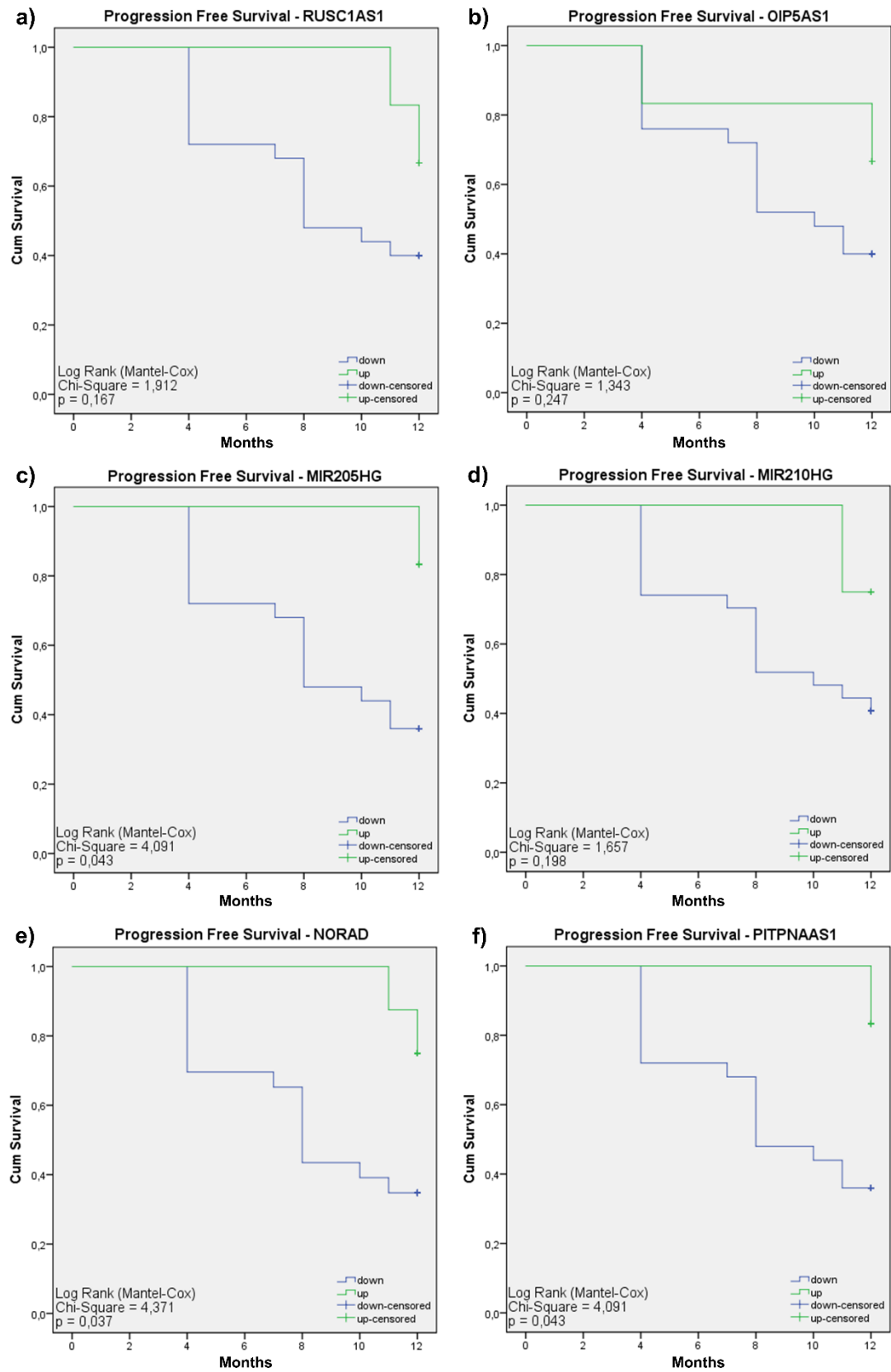


Figure 7



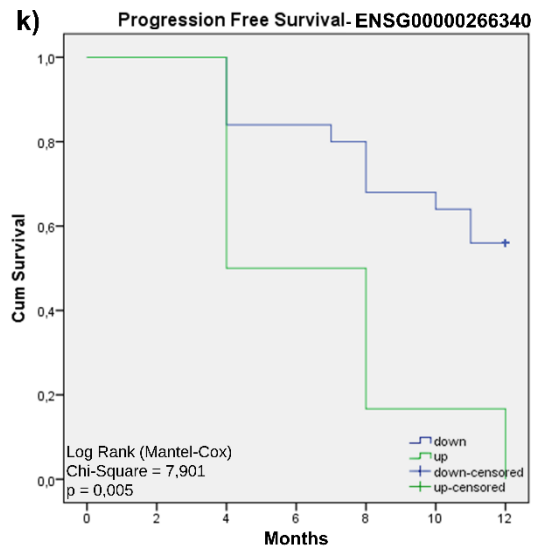
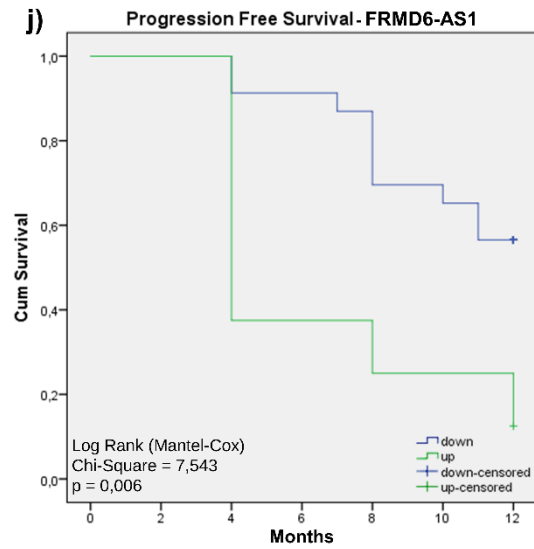
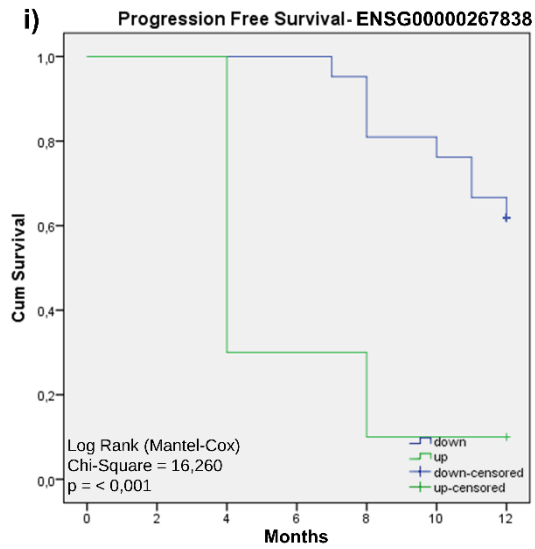
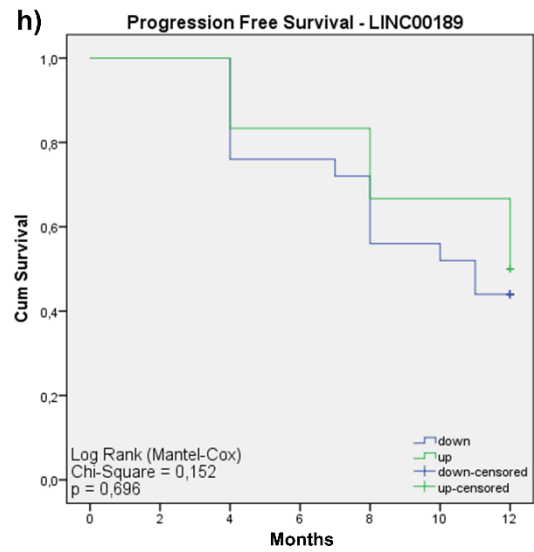
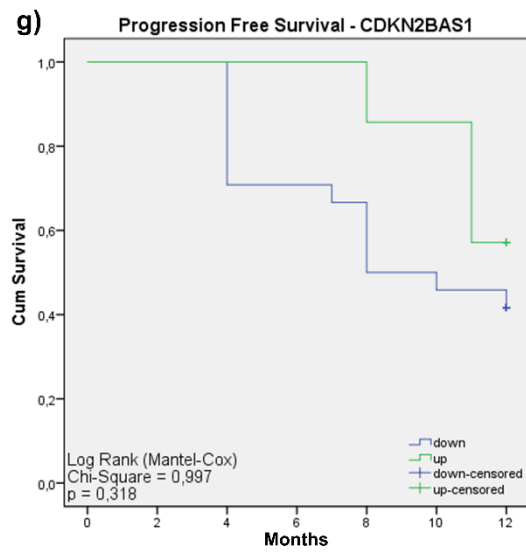
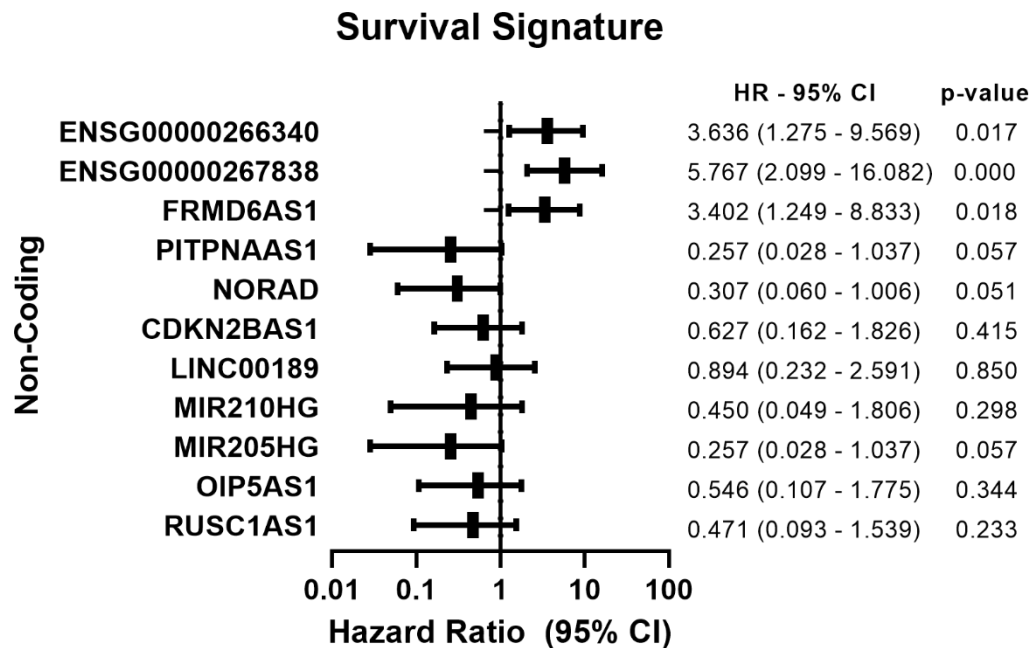


Figure 8



CONCLUSÃO

Neste estudo, foi evidenciada a capacidade dos lncRNAs de modular a resposta terapêutica por meio de perfis de expressão. A assinatura gênica gerada por sua expressão auxilia no reconhecimento de pacientes em relação à resposta ao tratamento, consolidando seu potencial como biomarcadores para diagnóstico, prognóstico e monitoramento da evolução do CCU. Três lncRNAs se destacaram na análise de árvore de decisão, ENSG00000267838, FRMD6-AS1 e ENSG00000266340, sendo considerados discriminantes para classificar grupos de pacientes R e NR, mediante regulação positiva ou negativa.

Além disso, a análise de sobrevida livre de progressão revelou que a superexpressão desses lncRNAs está associada a um substancial aumento no risco de progressão da doença. Em particular, o lncRNA ENSG00000267838 mostrou notável capacidade de distinguir entre os grupos de pacientes R e NR. Ele apresenta altos níveis de expressão em tecidos normais do sistema reprodutor feminino e baixa expressão no CCU, além de interações significativas com miRNAs relevantes para o CCU, como miR-10-5p, miR-124-3p e miR-128-3p, e com a proteína DROSHA, associada à progressão desse câncer. Os lncRNAs MIR205HG, NORAD e PITPNA-AS1 foram identificados como relevantes para o CCU, desempenhando papéis significativos na progressão tumoral, metástase e resposta ao tratamento.

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