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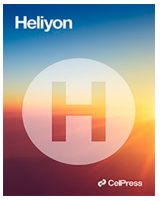

Research article

Potential new cancer biomarkers revealed by quantum chemistry associated with bioinformatics in the study of selectin polymorphisms

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ABSTRACT

Understanding the complex mechanisms involved in diseases caused by or related to important genetic variants has led to the development of clinically useful biomarkers. However, the increasing number of described variants makes it difficult to identify variants worthy of investigation, and poses challenges to their validation. We combined publicly available datasets and open source robust bioinformatics tools with molecular quantum chemistry methods to investigate the involvement of selectins, important molecules in the cell adhesion process that play a fundamental role in the cancer metastasis process. We applied this strategy to investigate single nucleotide variants (SNPs) in the intronic and UTR regions and missense SNPs with amino acid changes in the *SELL, SELP, SELE*, and *SELPLG* genes. We then focused on thyroid cancer, seeking these SNPs potential to identify biomarkers for susceptibility, diagnosis, prognosis, and therapeutic targets. We demonstrated that *SELL* gene polymorphisms rs2229569, rs1131498, rs4987360, rs4987301 and rs2205849; *SELE* gene polymorphisms rs1534904 and rs5368; rs3917777, rs2205894 and rs2205893 of SELP gene; and rs7138370, rs7300972 and rs2228315 variants of SELPLG gene may produce important alterations in the DNA structure and consequent changes in the morphology and function of the corresponding proteins. In conclusion, we developed a strategy that may save valuable time and resources in future investigations, as we were able to provide a solid foundation for the selection of selectin gene variants that may become important biomarkers and deserve further investigation in cancer patients. Large-scale clinical studies in different ethnic populations and laboratory experiments are needed to validate our results.

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1. Introduction

It is estimated that by 2024, cancer will account for around 1680 fatalities per day in the United States (US), making it the second most common cause of death behind heart disease [1]. The age-standardized rates of thyroid cancer incidence were 3.1 per 100,000 men and 10.1 per 100,000 women [2]. Despite much recent progress, we still still don't fully understand how cancer develops and progresses.

Cell adhesion molecules (CAMs) are transmembrane proteins that play important roles in cell-cell communication and interaction [3–5]. Selectins are a family of CAMs that are involved in the initial steps of cell adhesion because they can bring cells closer together. This is important for the immune response, as it allows leukocytes to move from the blood vessels to sites of infection or injury in the tissues [5–8]. The interaction between selectins and carbohydrates is weak and reversible allowing cells to adhere and detach from each other quickly and efficiently [4,6,9,10]. Thus, in addition to their role in the immune response, selectins have been implicated in other processes, including tumor metastasis [11,12]. The recognition of the important role of selectins has allowed the development of targeted drugs that may become important in the treatment of various conditions [13,14].

There are three types of selectins: L-selectin, E-selectin, and P-selectin [7,9,10]. L-selectin is encoded by the *SELL* gene located on the long arm of chromosome 1 (1q24.2) [15]. The glycosylation patterns of this protein may dictate its functions in the cell, but the mechanisms involved in these functions remain unclear [15]. Initially, its expression was considered exclusive to the leukocyte surface [5,15]; however, more recently, several groups, including ours [16], demonstrated its expression in several types of cancer and other cell types [11,12,17–22]. Overall, L-selectin favors interactions that allow both leukocytes [5] and metastatic tumor cells [17–20] to leave the bloodstream, come into contact with activated endothelial cells, and start the rolling process [5,10,21].

E-selectin is also expressed in endothelial cells and is involved in the recruitment of immune cells to sites of inflammation [7]. It is encoded by the *SELE* gene, which is located on the long arm of chromosome 1 (1q24.2). E-selectin plays an important role in inflammation by facilitating the adhesion and migration of immune cells to sites of inflammation [22,16]. Recent studies have shown that E-selectin may also be involved in cancer, as it was found to be upregulated in various types of cancer [23], including breast [24–26], lung [27,28], and pancreatic cancers [29], and its expression levels are associated with more aggressive tumors and poorer prognosis in cancer patients [30].

P-selectin has also been suggested to play a role in cancer [24,31] by promoting the spread of cancer cells by facilitating their migration using the CAM migration system. The protein is encoded by the *SELP* gene located on chromosome 1 (1q24.2). It plays a role in the process of blood clotting and inflammation and is found on the surfaces of platelets and endothelial cells [8,32,33]. In addition, P-selectin has been shown to be involved in the recruitment of immune cells to tumor sites [11,34]. One of the most important ligands of P-selectin is PSGL-1 (P-selectin glycoprotein ligand-1), a protein encoded by the *SELPLG* gene located on chromosome 12 (12q24.11). Recent studies [35,36] have shown that PSGL-1 may promote cancer progression by promoting the adhesion and migration of immune cells to the tumor microenvironment. This can lead to the recruitment of pro-tumor immune cells and suppression of anti-tumor immune responses, ultimately promoting tumor growth and metastasis [37]. PSGL-1 has been found to be upregulated in various types of cancer, and it is associated with more aggressive tumors and poorer prognosis [37–39]. In addition, targeting PSGL-1 is considered a potential approach for cancer immunotherapy [14,35,40–48].

Single nucleotide polymorphisms (SNP) are variations of DNA that cause a single nitrogenous base substitution in the gene sequence. Most SNPs are neutral, but some may contribute to disease predisposition, consequently acting as genetic markers, or serve as diagnostic biomarkers; influence disease evolution and help in the follow-up, management, or prognosis of the patient; and may even determine response to the treatment of conditions such as cancer [49]. The main advantages of using SNPs as biomarkers are related to their stability, as they have a low mutation rate and high frequency, are present in more than 1% of the population, and facilitate the optimization of analysis techniques through automation [50,51].

Depending on their location, SNPs can promote nucleotide substitutions that lead to different alterations in the DNA, modifications in the formation of proteins (to their structure, function, and stability), variations in protein formation (regarding its structure, function, and stability), regulation of protein-protein interactions, changes in the mechanisms of splicing, transcription, localization, and degradation of mRNAs, and functional changes in transcription factor binding sites, intron/exon splicing sites, exonic splicing promoter sites, and miRNA binding sites [52–54].

Understanding the complex mechanisms involved in diseases caused by or related to SNPs [50,51] has led to the development of several tests currently in use. However, the increasing number of described variants makes their study difficult and poses challenges for validation. Furthermore, most SNPs lack sufficient evidence linking them to human diseases, complicating the identification of variants worthy of further investigation or validation in patient cohorts. However, validation using patient samples can be expensive and time-consuming, and when not properly designed, it may not produce relevant results. Bioinformatics instruments help in the selection of SNPs that can influence the genesis and progression of diseases with greater probability [55–63]. Quantum chemistry aims to accurately describe the various chemical and physical properties of molecules [64]. Computational analysis methods based on molecular quantum chemistry allow the understanding, modeling, and prediction of molecular properties and their reactions in processes that occur in biological systems [64–72]. We combined bioinformatics and molecular quantum chemistry methods using different analytical algorithms to investigate the involvement of selectins in cancer. This strategy aims to provide a comprehensive view of the effects of amino acid changes on protein structure, including considerations of functional, structural, and stability impacts, as well as interactions with adjacent amino acids. We applied this unique innovative model strategy to investigate the potential roles of SNPs in *SELL, SELP, SELE*, and *SELPLG* in the carcinogenic process. We then focused on thyroid cancers, seeking their potential as biomarkers of susceptibility, diagnosis, prognosis, and therapeutic targets.

2. Materials and methods

We present in Fig. 1 the various stages of the development and use of this model in the study of the investigated selectins.

We used dbSNP from NCBI (http://www.ncbi.nlm.nih.gov/snp/) to retrieve the nsSNPs of interest.The selection was carried out considering a minor allele frequency (MAF) > 0.1, aiming to facilitate subsequent population validation. The gene sequences and FASTA sequences of the proteins were obtained from Genome Browser (https://genome.ucsc.edu/cgi-bin/hgGateway) and Universal Protein Resource – UniProt [73] (https://www.uniprot.org/; *SELL* ID: P14151, *SELE* ID: P16581, *SELP* ID: P16109 and *SELPLG* ID: Q14242), respectively.

2.1. Analysis of gene sequence

All nsSNPs with MAF>0.1 were analyzed using PredictSNP2.0 [56] (https://loschmidt.chemi.muni.cz/predictsnp2/). This software consists of five tools that analyze the impact of nucleotide substitutions on DNA: CADD [74] associates the presence of SNPs with deleterious functions (insertion or deletion) in the human genome; DANN [75] uses a Neutral Network for deleterious gene sequence annotations; FATHMM [76] predicts possible functional consequences caused by the presence of SNPs in coding and non-coding regions; FunSeq2 [77] prioritizes the analysis of somatic alterations related to the appearance of neoplasms; and GWAVA [78] evaluates the functional impact of changes in non-coding regions. In addition, we used PredictSNP2.0 [56] to complement the computational analyses with experimental annotations from eight databases (ClinVar, dbSNP, Ensembl Genome Browser, GenBank, HaploReg, OMIM, RegulomeDB, and UCSC Genome Browser), enabling the correlation of the data obtained in silica with the existing literature available in these databases.

2.2. Analysis of missense SNPs with amino acid change

We employed PredictSNP1.0 [57] (https://loschmidt.chemi.muni.cz/predictsnp1/) to perform a morphofunctional analysis of proteins with amino acids altered by the presence of missense SNPs. PredictSNP1.0 [57] comprises eight tools: SIFT [79], which performs an estimation of the effects of amino acid substitution on protein function based on homology and the chemical characteristics of the amino acids; PolyPhen-1 [80] and PolyPhen-2 [81], which assesses the impacts on protein structure and function by empirical methods of analysis and also by comparison of physical properties of the molecules; MAP [82] evaluates the physicochemical variations of the protein; PhD-SNP [62] uses Support Vector Machine (SVM) methodology for protein structure and sequence analysis; SNAP [60] evaluates changes in the secondary structure of the protein, as well as compares solvent accessibility in case of amino acid changes by Neural Network methodology; PANTHER [61] evaluates protein function; and nsSNPAnalyzer [55], which uses Random Forest methodology with sequence alignment and 3D structure to assess phenotypic impacts. MuPRO [63] (http://mupro.proteomics.ics.uci.edu/) was used to evaluate protein stability.

2.3. Molecular docking

We used the molecular docking technique to evaluate how P-selectin, both in its wild form and in different forms with possible amino acid changes, interacts with its ligand PSGL-1. All possible combinations resulting from the presence of genetic polymorphisms

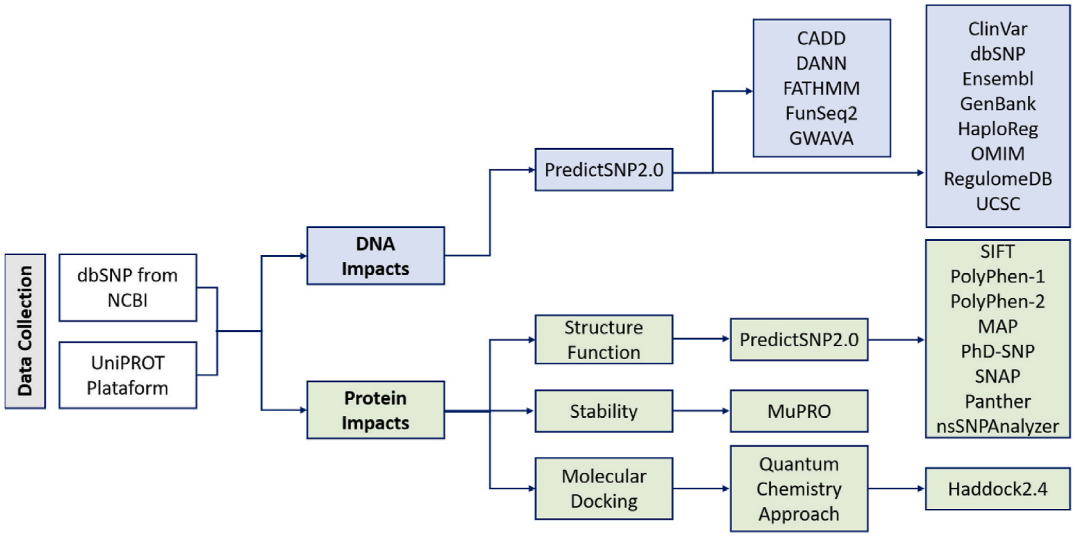

Fig. 1. Study model development steps from publicly available datasets, open-source bioinformatics tools, and molecular quantum chemistry methods to explore the role of selectins variants in thyroid cancer.

Table 1
In silico analysis of SNPs in *SELL*, *SELP*, *SELPLG* and *SELE* genes considering DNA alterations evaluated by PredictSNP2.0.

	Position	ID	Ref	Alt	Classification	PredictSNP2.0	CADD	DANN	FATHMM	FUNSEQ2	GWAVA
SELL	169695726	rs4987360	A	G	intronic	**D**	**D**	**D**	?	**D**	N
Chr #1	169704697	rs2229569	G	A	exonic	**D**	**D**	**D**	**D**	N	?
	169706069	rs4987301	G	A	intronic	**D**	**D**	**D**	**D**	**D**	N
	169706069	rs4987301	G	T	intronic	**D**	**D**	**D**	**D**	**D**	N
	169707345	rs1131498	A	G	exonic	N	**D**	N	N	N	N
	169712216	rs2205849	T	C	upstream	**D**	**D**	**D**	**D**	**D**	**D**
SELE **Chr #1**	169727805	rs5368	G	A	exonic	N	N	N	N	**D**	**D**
	169729684	rs1534904	T	A	intronic	**D**	**D**	**D**	?	**D**	N
	169729684	rs1534904	T	G	intronic	N	**D**	N	N	**D**	N
SELP	169596108	rs6133	C	A	exonic	N	N	N	**D**	N	**D**
Chr #1	169596108	rs6133	C	G	exonic	N	N	N	**D**	N	**D**
	169597075	rs6127	C	G	exonic	N	N	N	N	N	?
	169597075	rs6127	C	T	exonic	N	N	N	N	N	?
	169601781	rs3917777	T	A	intronic	**D**	**D**	**D**	?	**D**	N
	169601781	rs3917777	T	C	intronic	**D**	**D**	?	?	**D**	N
	169601781	rs3917777	T	G	intronic	**D**	**D**	**D**	?	**D**	N
	169605484	rs2205894	T	A	intronic	**D**	**D**	**D**	**D**	?	N
	169605484	rs2205894	T	G	intronic	**D**	**D**	**D**	**D**	?	N
	169605486	rs2205893	T	A	intronic	**D**	**D**	**D**	**D**	?	N
	169605486	rs2205893	T	G	intronic	**D**	**D**	**D**	**D**	?	N
	169611647	rs6131	C	T	exonic	N	N	N	N	N	N
SELPLG	108623488	rs7300972	T	A	exonic	N	N	N	N	**D**	**D**
Chr #12	108623488	rs7300972	T	C	exonic	N	N	N	N	**D**	**D**
	108623898	rs201851784	A	G	exonic	N	N	N	N	N	**D**
	108623898	rs201851784	A	T	exonic	N	N	N	N	N	**D**
	108624122	rs2228315	C	T	exonic	N	N	N	N	N	N
	108628692	rs7138370	G	A	intronic	N	**D**	**D**	N	**D**	**D**

Abbreviations: Chr-Chromossome; ID–Identification of the SNP; Ref–Reference allele; Alt–Altered allele; N–Neutral; D-Deleterious and ?– Unknown.

were evaluated. The Haddock 2.4 server [83,84] was used to rank the complexes of interaction, and a literature search was carried out to identify important amino acid residues for interaction between P-selectin and PSGL-1. Three residues were found for each protein (P-selectin - Gln61, Asn62, and Arg126; PSGL-1 - Tyr46, Tyr48, and Tyr51). Thus, to evaluate the structural behavior of the complexes based on the structural changes caused by amino acid substitutions, the hydrogen bonds between the main amino acid residues of the two proteins were evaluated.

3. Results

3.1. L-selectin (SELL gene)

We were able to recover 216 polymorphisms with MAF>0.1 from dbSNP. However, after excluding repeats and merged records, only 55 unique polymorphisms and 80 nucleotide changes remained. The SNPs were classified as upstream variant (4), intronic (46), 3′UTR (2), and exonic (3, including 2 nonsynonymous and 1 synonymous). All 80 alterations were evaluated using PredictSNP2.0. A total of 38 variants (47.5%) were considered neutral for all tools in consensus. Five (6.3%) SNPs were considered deleterious in at least four of the six tools (Table 1). rs4987360 is an intronic SNP that changes adenine to guanine (A/G). This alteration was considered deleterious by four (66.7%) tools, suggesting possible structural and functional DNA changes. Another polymorphic variant, rs2229569, is an exonic SNP that promotes the exchange of a guanine for an adenine (G/A) or thymine (G/T). Both these alterations can promote amino acid changes in the corresponding proteins. The G/A alteration was considered deleterious in four (66.7%) PredictSNP2.0 tools suggesting that it is capable of altering DNA structure and folding. In addition, it promotes the amino acid switch from proline to serine at position 213 (P213S). P213S alterations were considered deleterious by the MAPP tool, suggesting physicochemical alterations in the protein structure. Decreased stability was observed (ΔΔG = −0.6147; MuPRO), but there was no significant alteration in the pattern of ligation with adjacent amino acids (Dynamut2.0). The G/T alteration was considered deleterious in 3 (50.0%) PredictSNP2.0 tools. This variant promoted the exchange of proline for threonine at position 213 (P213T). P213T alteration was considered deleterious by the MAPP, PhdSNP, and SIFT tools (Table 2), suggesting physicochemical, structural, and functional alterations in the corresponding protein. Decreased stability (ΔΔG = −0.6483; MuPRO) and rigidity of the structure (ΔΔSvibENCoM: −0.048 kcal.mol-1.K-1; Dynamut2.0) were observed; however, this change did not produce significant alterations in the binding patterns with adjacent amino acids.

rs4987301 is an intronic SNP with two possible nucleotide alterations (G/A and G/T). Both alterations were considered deleterious for all PredictSNP2.0 tools (Table 1), except GWAVA, suggesting an important role in DNA modification considering the structure, pattern of interaction with adjacent nucleotides, and function. Similarly, rs2205849 is an upstream SNP that promotes the change from thymine to cytosine in the DNA structure and was considered deleterious in all PredictSNP2.0 tools (Table 1).

rs1131498 is an exonic polymorphism that causes the exchange of adenine for guanine (A/G). This alteration is considered deleterious in CADD, suggesting that it has harmful effects on the human genome (PredictSNP2.0). The consequence of A/G alteration in the protein is a change of phenylalanine to leucine at 193 position (F193L). F193L alteration was found to be neutral in all PredictSNP1.0 tools (Table 2); however, it decreased stability (ΔΔG = −0.3179; MuPRO) and increased molecule flexibility (ΔΔSvibENCoM: 0.005 kcal.mol-1.K-1; Dynamut2.0) were observed. Furthermore, a different pattern of interaction with adjacent amino acids was observed with the loss of hydrophobic contact, probably explaining the changes in flexibility, as shown in Fig. 2A (Dynamut2.0).

3.2. E-selectin (SELE gene)

Data obtained from dbSNP indicated 97 polymorphisms with MAF >0.1. However, some records were merged, and after deleting repeats, we observed 32 unique polymorphisms and 58 nucleotide alterations. The SNPs were classified according to their position in DNA: upstream variant (5), downstream variant (3), 3′UTR (2), intronic (20), and exonic (2, including 1 synonymous and 1 nonsynonymous). All 58 nucleotide alterations were evaluated by the PredictSNP2.0, and 82.7% (n = 48) were considered neutral in all tools, but one (rs1534904) was considered deleterious in four out of five tools, as presented in Table 1.

Table 2
Bioinformatics analysis of SNPs that cause amino acid alterations in L-selectin (*SELL*), E-selectin (*SELE*), P-selectin (*SELP*) and PSGL-1 (*SELPLG*) proteins according to PredictSNP1.0.

Gene	ID	AA Change	PredictSNP1.0	MAPP	PhD-SNP	PolyPhen-1	PolyPhen-2	SIFT	SNAP
SELL	rs1131498	F193L	N	N	N	N	N	N	N
	rs2229569	P213S	N	**D**	N	N	N	N	N
	rs2229569	P213T	N	**D**	**D**	N	N	**D**	N
SELE	rs5368	H468Y	N	N	N	N	N	N	**D**
SELP	rs6131	S331 N	N	N	N	N	N	N	N
	rs6127	D541 N	N	N	N	N	N	N	N
SELPLG	rs2228315	M62I	N	N	N	**D**	N	N	N
	rs201851784	V137A	N	N	N	N	N	N	N
	rs7300972	M274V	N	**D**	N	N	N	**D**	N

Abbreviations: ID-Identification of the SNP; AA-Amino Acid; N-Neutral; D-Deleterious.

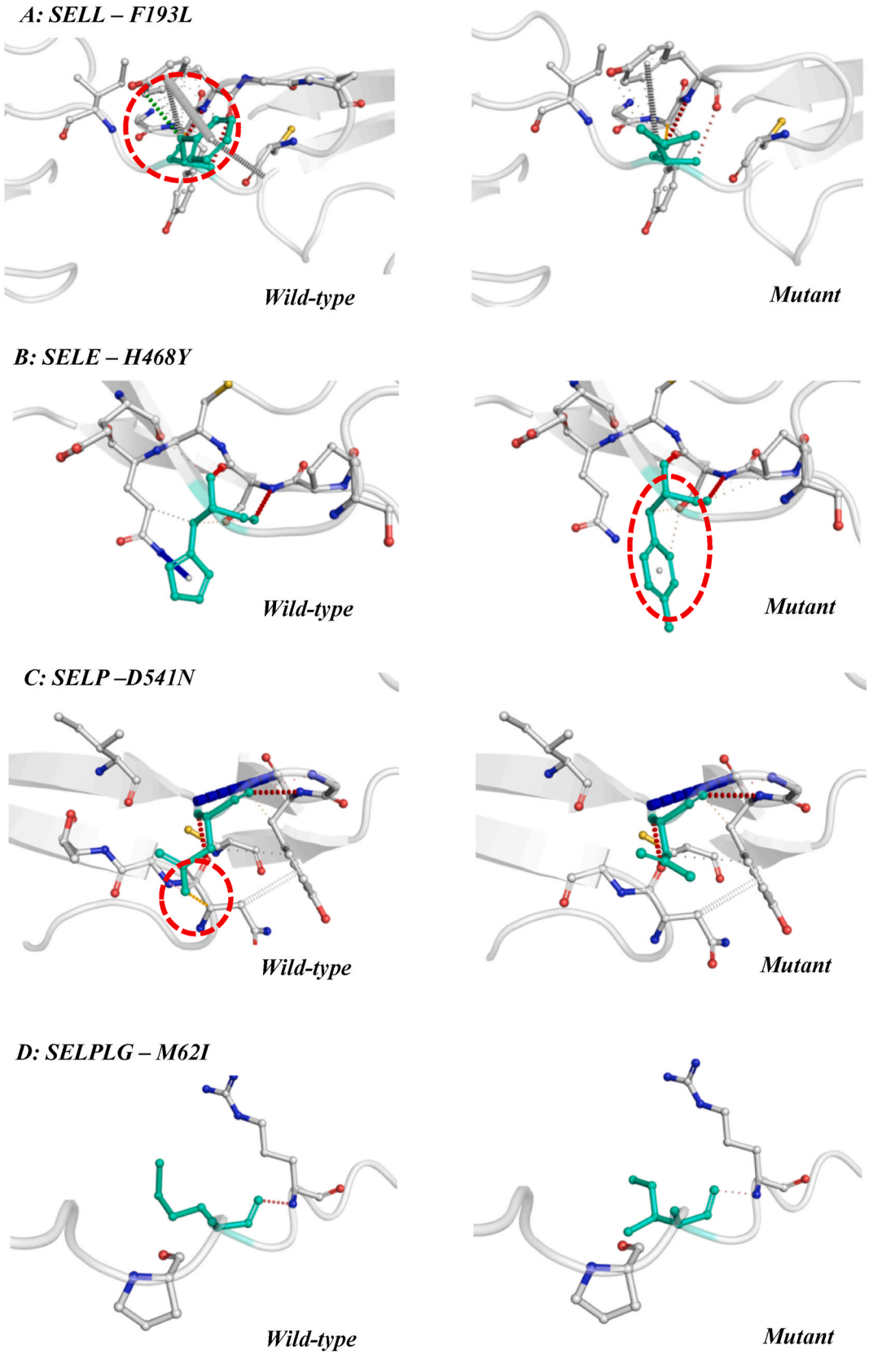

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Fig. 2. Interatomic changes observed between adjacent amino acids by Dynamut2.0. A: The loss of hydrophobic contact caused by rs1131498 (F193L) in the *SELL* gene causes a different pattern of interaction with adjacent amino acids.; B: the *SELE* gene's rs5368 (H468Y) promotes modifications to hydrogen bonding with adjacent amino acids; C: rs6127 (D541 N) of the *SELP* gene causes changes in the binding pattern with adjacent amino acids due to the disappearance of the hydrogen bond and D: rs2228315 (M62I) of the *SELPLG* gene promotes changes in the amino acid binding pattern leading to a weaker water mediated hydrogen bonding.

rs1534904 is an intronic SNP with two possible nucleotide alterations, T/A and T/G. T/A exchange was considered deleterious in 4 (66,7%) of the tools evaluated (Table 1), suggesting possible structural, functional, and damage control pathway alterations in DNA. The T/G alteration was considered deleterious in 2 (33,4%) of the tools employed (Table 1), suggesting a deleterious effect on the human genome.

rs5368 is the only exonic SNP with a MAF>0.1 recorded for the SELE gene in the dbSNP database. It is an exchange of a guanine for an adenine in the DNA structure that is considered deleterious by two out of the six PredictSNP2 tools. This polymorphism promotes the exchange of histidine with a tyrosine at position 468 (H468Y). It was evaluated using PredictSNP1.0 (Table 2) and was considered

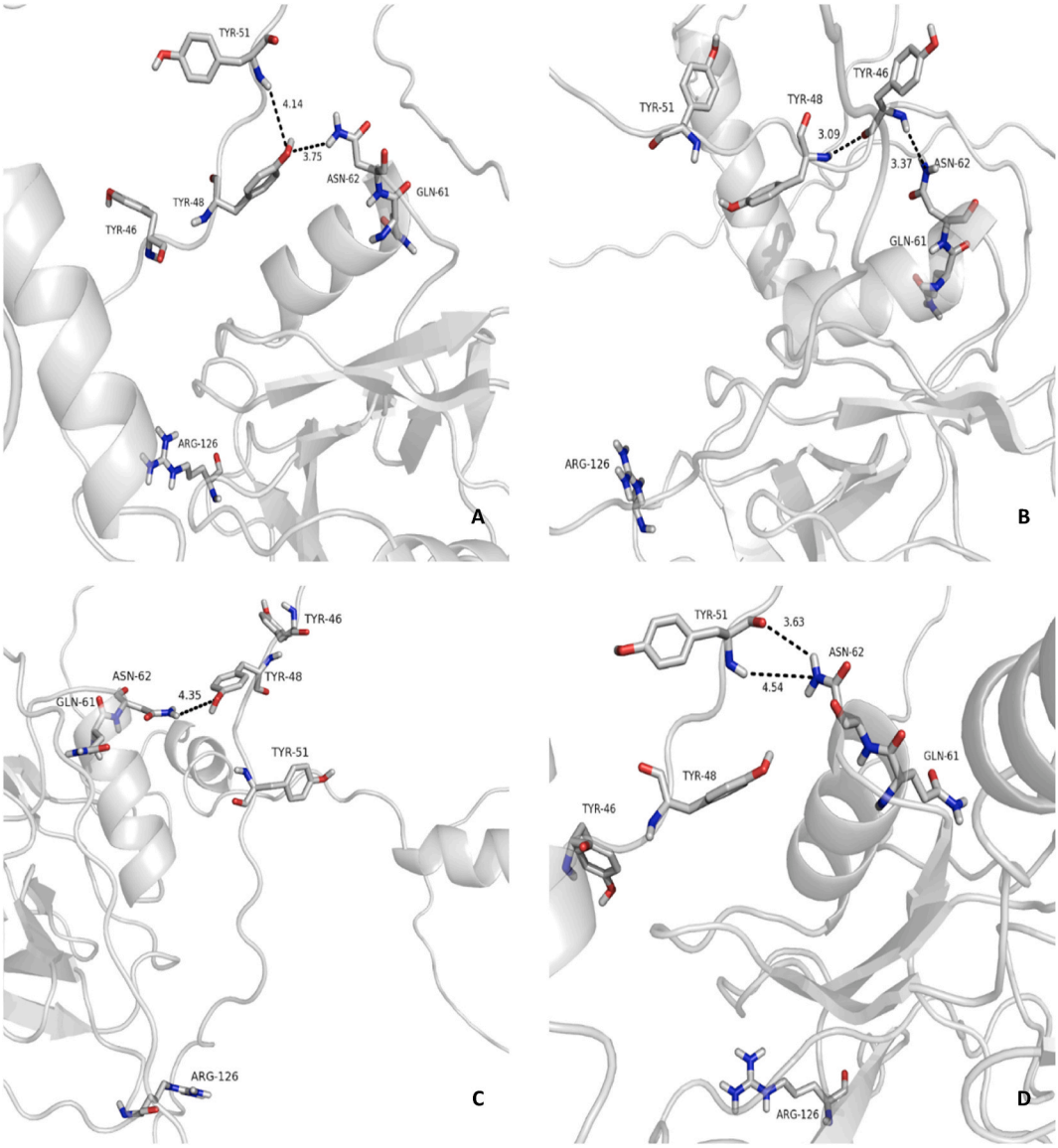

Fig. 3. Molecular docking analysis of the interaction between P-selectin and PSGL-1. A: P-selectin and PSGL-1 in the wild type form. B: P-selectin in wild form and PSGL-1 with M62I substitution showing the change in the hydrogen bond profile, as this change brings the two molecules closer together. C: P-selectin with D541 N substitution and PSGL-1 in wild type form showing the loss of hydrogen bonds and D: Docking analysis of both altered molecules: P-selectin with D541 N substitution and PSGL-1 with M62I substitution.

harmful only by the SNAP tool, indicating that it could cause changes in the secondary structure of the E-selectin protein (Table 1). This exchange is not located in the region of post-translational modifications according to the MutPred2 tool. Increased protein stability (ΔΔG = −0.1524, MuPRO) and increased molecular flexibility (ΔΔSVibENCoM: 0.024 kcal.mol-1.K-1; Dynamut). Changes in the hydrogen bonds with adjacent amino acids were observed (Fig. 2B).

3.3. P-selectin (SELP gene)

We retrieved 245 polymorphisms that presented MAF>0.1 from dbSNP, but after removing the repeats, 88 unique polymorphisms and a total of 144 nucleotide changes remained. The SNPs were classified as upstream variant (4), 3′UTR (1), intronic (77), and exonic (6, including 3 synonymous and 3 non-synonymous).

All 144 alterations were evaluated using PredictSNP2.0. A total of 108 (75.0%) were considered neutral in all tools in the consensus, but six (4.2%) were identified as deleterious in at least four of the five tools, as presented in Table 1.

rs3917777 (T/A, T/C, and T/G), rs2205894 (T/A and T/G), and rs2205893 (T/A and T/G) were intronic SNPs and were considered deleterious in four out of the 5 PredictSNP2.0 tools (Table 1). rs6127 (C/T; D541 N) and rs6131 (C/T; S331 N) were identified as neutral in all PredictSNP2.0 tools (Table 1). However, these are exonic SNPs with amino acid changes in their protein structure that can cause morphofunctional modifications. Therefore, we proceeded with PredictSNP1.0, MuPRO, and Dynamut evaluation and observed that rs6127 (C/T; D541 N) and rs6131 (C/T; S331 N) were considered neutral in all PredictSNP1.0 tools (Table 2), suggesting no alteration in the structure or function of E-selectin protein. However, these SNPs cause decrease in protein stability (ΔΔG = −0.7148 and ΔΔG = −0.1887, respectively; MuPRO).

rs6127 (C/T; D541 N) promotes a change in the binding pattern with adjacent amino acids due to the disappearance of a hydrogen bond (Fig. 2C), leading to decreased stability and flexibility of the protein structure (ΔΔSVibENCoM: 0.643 kcal.mol-1.K-1; Dynamut).

3.4. PSGL-1 (SELPLG gene)

Ninety-five SNPs (MAF>0.1 were available in the dbSNP database; however, only 29 SNPs and 48 nucleotide alterations were observed after the removal of duplicate records. The SNPs were classified as follows: downstream variant (1), upstream variant (3), 3′-UTR (1), intronic (21), and exonic (3).

All 48 alterations were evaluated using PredictSNP2.0. Seventeen (35.4%) alterations were considered neutral in all tools, and only rs7138370 was considered deleterious in four of the six tools, suggesting its possible role in DNA structural and functional alterations (Table 1).

In addition, we studied the exonic SNPs rs2228315 (C/T; M62I), rs7300972 (T/C; M274V), and rs201851784 (A/G; V137A) (Table 2). rs2228315 (C/T; M62I) was considered neutral by PredictSNP2.0, but it was found to be deleterious using the PolyPhen-1 tool (PredictSNP1.0), and MuPRO analysis indicated that this SNP can decrease protein stability (ΔΔG = −0.5872). In addition, it may cause rigidification of the protein structure (ΔΔSVibENCoM: −0.133 kcal.mol-1.K-1; Dynamut) and alterations in amino acid-binding patterns, leading to weaker water-mediated hydrogen bonding (Fig. 2D). rs7300972 was considered deleterious by FUNSEQ and GWAVA tools (PredictSNP2.0 – Table 1), suggesting a possible role in somatic alterations and modifications in DNA structure. This SNP was considered deleterious by the MAPP and SIFT tools (Table 2), which revealed putative structural and physicochemical alterations. rs201851784 (A/G; V137A) was considered neutral by all the tools employed (PredictSNP1.0; Table 2 and PredictSNP2.0; Table 1).

3.5. Molecular docking analysis

We evaluated the interaction between P-selectin and PSGL-1, considering the amino acids reported in the literature as relevant to these protein interactions. Hydrogen bonds were observed between residues Gln61, Asn62, and Arg126 for P-selectin, and Tyr46, Tyr48, and Tyr51 for PSGL-1. Twenty-eight combinations were tested, taking into account all the possibilities arising from the presence of rs6131 (S331 N) and rs6127 (D541 N) in *SELP* and rs2228315 (M62I), rs201851784 (V137A), and rs7300972 (M274V) in *SELPLG*.

The interaction between P-selectin and PSGL-1 in the wild-type form showed the presence of a hydrogen bond between Tyr48 and Asn62 at a distance of 3.75A and a hydrogen bond between Tyr48 and Tyr51 at a distance of 4.14A (Fig. 3A). The M62I substitution in PSGL-1 caused a change in the hydrogen bond profile, in which case there was a bond between Tyr48 and Tyr46 3.09A and a bond between Tyr46 and Asn62 of 3.37A (Fig. 3B). However, when the D541 N substitution occurs in P-selectin, this pattern results in only one hydrogen bond between Tyr48 and Asn62 of 4.35A (Fig. 3C). The simultaneous presence of both alterations (P-selectin - D541 N and PSGL-1 - M62I) causes the presence of two hydrogen bonds between Tyr51 and Asn62 (3.63A and 4.54A - Fig. 3D).

4. Discussion

Selectins have long been thought to be specific to the immune system or correlated cells. However, more recent solid evidence has demonstrated their overexpression in tumor cells, suggesting that these CAMs play an important role in metastatic pathways [85]. To better understand their role in cancer and seek selectin variants that can be used as thyroid cancer biomarkers, we developed a model based on a wide repertoire of bioinformatics and quantum chemistry analysis tools. We identified a set of SNPs that may be considerably prevalent in the population and connect with multiple diseases, including cancer, because their MAF>0.1. We demonstrated that rs2229569, rs1131498, rs4987360, rs4987301 and rs2205849 of *SELL* gene; rs3917777, rs2205894 and rs2205893 of *SELP* gene; rs7138370, rs7300972 and rs2228315 of *SELPLG* gene and; rs1534904 and rs5368 of *SELE* gene may be useful biomarkers of diseases,

particularly in cancer patients, considering the role of the examined selectins in these conditions.

L-selectin (*SELL*) expression has been associated with several types of cancers, including endometrial [86,87], breast [26,88], and thyroid [18,19,89]. An in silico study [88] using TCGA and On-comine databases found higher expression of *SELL* in tumor tissues, suggesting that L-selectin could be a biomarker of the inflammatory microenvironment. In addition, the authors observed higher *SELL* expression in patients with breast cancer, with better outcomes. Kobawala et al. [18] analyzed 150 patients with thyroid nodules (83 papillary thyroid carcinomas and 67 benign nodules) using ELISA and immunohistochemistry techniques and observed a higher protein expression of L-selectin in cells and higher serum levels in PTC patients than in benign thyroid diseases, suggesting a possible role of this adhesion molecule in the development of thyroid cancer. Despite several studies reporting the gene and protein expression of L-selectin, the literature is still scarce with regard to the study of its polymorphisms in cancer. We demonstrated that rs2229569 (exonic; G/A - P213S and G/T - P213T) may alter the structure and folding of DNA and promote physicochemical alterations in protein structure with decreased stability and possible protein functional alterations. Similarly, rs1131498 was shown to modify binding patterns with adjacent amino acids, thus altering protein structure at the molecular and atomic levels. We also did not find reports of other possible cancer biomarkers in the literature, such as the intronic polymorphisms rs4987360 (A/G), rs4987301 (G/A and G/T), and rs2205849 (T/C), which were considered deleterious by all the tools that we used.

Expression of E-selectin at significantly higher levels has been related to several types of cancer, such as colorectal [11], gastric [90], and breast [24–26], and was correlated with a decreased risk of hospitalization or need for respiratory support/death in COVID-19 cases [91]. Na Li et al. [92], in an in-silico study employing TCGA and the GEPIA server, found higher gene expression in tumor samples when compared to healthy samples, and in of lymph node metastasis in colorectal cancer [92]. Furthermore, when antitumor drugs were administered, gene expression levels were reduced, suggesting a possible role of E-selectin as an oncogene. Targeting E-selectin has emerged as a potential therapeutic strategy for cancer treatment [93–95]. Several approaches have been developed to target E-selectin, including monoclonal antibodies and small-molecule inhibitors, and have shown promise in preclinical studies by reducing tumor growth and metastasis [96–100]. *SELE* polymorphisms have been reported as possible risk indicators for several medical conditions such as hypertension in cases of occupational stress [101], risk of coronary artery disease [102], renal cyst enlargement in patients with polycystic kidney disease [103], type 2 diabetes [104], subclinical atherosclerosis, and increased platelet activity in systemic lupus erythematosus [105]. We demonstrated that rs1534904 (T/A and T/G) can provoke important alterations in DNA. This polymorphism has not yet been reported in studies evaluating various disease conditions. We also showed that rs5368 (H468Y) may alter the secondary structure of E-selectin and is capable of increasing protein stability and flexibility. Zakariya BF et al. [25] found CT heterozygous genotype frequency significantly higher in breast cancer patients, confirming the importance of SELE polymorphisms in cancer risk prediction.

P-selectin (SELP) is mainly expressed on the surfaces of activated endothelial cells and platelets [6,32]. Its expression upregulation is correlated with the pathogenesis of various diseases, including atherosclerosis [106], thrombosis [107], diabetes [108] and cancer [11,24,31,34,109]. Some studies have suggested that SNPs of the SELP gene could be diagnostic biomarkers for head and neck [34] and pancreatic cancer [109]. This molecule has emerged as a potential therapeutic strategy for the treatment of various conditions, especially because of its important role in migration and metastatic events [11,24,31]. Our analysis also identified rs3917777 (T/A, T/C, and T/G), rs2205894 (T/A and T/G), and rs2205893 (T/A and T/G) as promising biomarkers that have not yet been investigated in patients with cancer. Molecular docking analysis demonstrated that the presence of rs6127 (D541 N) decreased the number of hydrogen bonds in this complex between P-selectin and PSGL-1, contributing to the distance between these proteins. P-selectin has been shown to promote cancer metastasis by facilitating the adhesion and migration of tumor cells [24,31]. The decrease in the number of hydrogen bonds in D541 N may be associated with the decrease in the formation of new metastatic sites.

PSGL-1 plays a key role in mediating leukocyte adhesion to activated endothelial cells and platelets, as well as facilitating leukocyte rolling and migration into sites of inflamation [41] and has been widely studied because of its role as an immune checkpoint and its promising role in immune checkpoint landscaping [35,41]. Monoclonal antibodies against PSGL-1 have been shown to inhibit tumor growth and metastasis in preclinical models of cancer [35,37,44]. However, studies on these polymorphisms and their possible clinical use are still scarce. Our data indicated that both rs7138370 and rs7300972 could promote DNA structural and functional alterations; rs2228315 (C/T; M62I) may also promote modifications in DNA and modify structure, function, stability, rigidification, and interaction with adjacent amino acids, making these SNPs interesting candidates for biomarkers. PSGL1 polymorphisms can help identify response patterns to immunological therapies to which this molecule is targeted, and improve the quality of treatment offered to cancer patients. Our molecular coupling analysis showed that the change in M62I can promote a rapprochement between P-selectin molecules and PSGL-1 owing to closer hydrogen bonds, which may facilitate the migratory process that depends on this interaction. Since higher expression of P-selectin is correlated with metastasis, alterations in its binding pattern with its natural ligand, PSGL-1, may influence the neoplastic migratory process. This molecular rapprochement may play a relevant role in the formation of metastatic sites, making the presence of rs2228315 a biomarker of cancer aggressiveness and contributing to the characterization and management of various tumor types.

5. Conclusions

We developed a model that has the advantage of using publicly available datasets and open source bioinformatics tools. This strategy will save valuable time and resources in future investigations as we were able to provide solid foundations in the selection of selectin gene polymorphisms that may become important biomarkers and deserve further investigation in cancer patients. Large-scale clinical studies in different ethnic populations as well as laboratory experiments may provide validation of our results.

The ability to assess the impacts of amino acid changes in protein structure promoted by the presence of genetic polymorphisms can provide valuable information about the genetic predisposition, diagnosis, prognosis and response to treatment, contributing to

personalized medicine and targeted therapies.

Ethical approval and consent to participate

Not applicable.

Availability of data and materials

The data presented in this study are available in this article.

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CRediT authorship contribution statement

Larissa Teodoro Rabi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Davi Zanoni Valente:** Writing – original draft, Validation, Methodology, Investigation. **Elisangela de Souza Teixeira:** Data curation, Conceptualization. **Karina Colombera Peres:** Writing – original draft, Visualization, Formal analysis, Conceptualization. **Michell de Oliveira Almeida:** Validation, Methodology. **Natassia Elena Bufalo:** Writing – original draft, Visualization, Supervision, Conceptualization. **Laura Sterian Ward:** Writing – review & editing, Visualization, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: All authors of this research paper declare not having any conflict of interest, except Dr Laura Ward, who is a member of Heliyon Cancer Research Editorial's Team. The authors have nothing else to disclose. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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