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In silico evaluation of the gene expression profile of syndecan-4 in different breast tumor subtypes

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ABSTRACT

Introduction: Breast cancer is one of the main causes of death in women. Luminal tumors A and B show good response with hormonal treatments, tumors that overexpress HER-2 can be treated with monoclonal antibodies, whereas triple-negative tumors have few treatments available because they present low or absent expression of hormone receptors and HER-2, in addition, they present worse tumor progression. Syndecans are heparan sulfate proteoglycans that have the function of interacting with growth factors, cytokines, and extracellular matrix, thus modulating important processes in tumor progression. **Objective:** Analyze the expression of syndecan-4 in different subtypes of breast tumors. **Methods:** Bioinformatics is a useful tool for the study of new biomarkers. In the present study, the TCGA database (514 patients) and Metabric (1,898 patients) were analyzed using the cBioportal software. Gene expression data were analyzed by RNA-Seq and Microarray from biopsies of breast tumors. **Results:** An alteration in syndecan-4 gene expression was observed among the different subtypes of breast tumors. Patients with a triple-negative tumor had decreased expression for syndecan-4 in both databases. **Conclusion:** Syndecan-4 is a potential biomarker for breast tumor prognosis since decreased expression of syndecan-4 is related to triple-negative breast cancer.

Keywords: computational biology; gene silencing; Heparan Sulfate Proteoglycans; breast neoplasms.

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INTRODUCTION

Breast cancer is one of the leading causes of death among women worldwide, in Brazil there were 15 deaths per 100,000 in 2019, and this type of cancer is responsible for 16.1% of deaths from oncological causes[1]. Breast tumors are classified according to the eighth edition of the American Joint Commission of Cancer (AJCC)[2], Based on this classification, it is possible to determine the prognosis more assertively. This classification is based on the division of breast tumors by histological and anatomical TNM analysis, where T is the volume of the tumor, N is the number of lymph nodes involved and M is the number of metastases. On the other hand, tumor-grade, biomarkers such as HER2 (Human Epidermal growth factor Receptor-type 2), estrogen receptors (ER),

progesterone receptors (PR), and multigene test panels (Oncotype DX) are additional determinants of this classification[3].

The choice of treatment between hormone therapy and systemic chemotherapy depends on anatomical and histological analysis and the analysis of biomarkers[3].

Based on the relevance of biomarkers for clinical and therapeutic determination, this study opted to deepen its studies using molecular subtypes as a classification. In the classification by molecular subtypes, tumors are divided exclusively according to the expression of biomarkers, which are estrogen, progesterone, and HER2 receptors[3].

Luminal A and B breast tumors have estrogen and progesterone receptors and are very responsive to hormonal treatments[4]. They are divided into luminal A if it is a slow-growing cell, and luminal B if it is a faster-growing cell. HER2+ breast cancer has overexpression of the HER2 receptor on the cell membrane. HER2-positive tumors grow faster than luminal tumors and respond to treatment with trastuzumab, an anti-HER2 monoclonal antibody[5]. The last subtype of breast cancer is known as triple-negative breast cancer. It is characterized by low or absent expression of hormone receptors and HER2. Due to the low expression of these receptors, the treatments described above do not work. The main treatment for this disease is chemotherapy[6], such as platinum (cisplatin or carboplatin) or PARP inhibitors (olaparib and talazoparib).[2].

Syndecans are heparan sulfate proteoglycans with a transmembrane domain. There are four syndecan members or isoforms, syndecans 1, 2, 3, and 4, all of which arose from the duplication and divergent evolution of a single ancestral gene. These proteins are linked to three to five heparan sulfate and chondroitin sulfate chains[7,8].

The syndecans are made up of glycosaminoglycan chains, heparan sulfate, and chondroitin sulfate, which help to interact with different growth factors and components of the extracellular matrix, modulating various processes such as cell proliferation and migration. For this reason, syndecans are of immense importance in tumor progression[7,8].

Syndecan-4 is encoded by the SDC4 gene, and among all the syndecans it has the widest distribution and is the only one consistently found in focal adhesions, being of immense importance in cytoskeleton organization and cell migration[9]. Despite the importance of syndecan-4 in tumor progression, the relationship between syndecan-4 and the tumor process is still unclear and the data is controversial.

In colorectal tumors, increased syndecan-4 expression is related to a worse patient prognosis, while in prostate tumors, increased syndecan-4 is related to a better prognosis[10,11].

This study aimed to evaluate, in silico, the expression of syndecan-4 in patients with breast tumors, as well as to analyze whether syndecan-4 could be a potential biomarker of different breast tumor subtypes for diagnostic purposes.

METHODS

Bioinformatics analysis

Data from the TCGA database and Metabric on syndecan-4 gene expression were analyzed using the cBioPortal software. (https://www.cbioportal.org/). The gene expression analyses used the data obtained by Microarray and RNA-seq available in the TCGA database. In addition to the gene expression data obtained by Microarray from the Metabric database. In both databases, the samples analyzed were breast tissues. The patients in the TCGA database (n=514) were divided into breast tumor subtypes: triple-negative (n=98), luminal A/B (n=358), and HER2+ (n=58). Patients from the Metabric database (n=1898), 1140 of whom were luminal A/B patients, 220 HER2+ patients, 398 triple-negative breast tumors, and 140 control patients.

Statistical analysis

GraphPad Prism software was used, and the t-test was used in analyses with 2 groups. ANOVA was used in analyses with more than 2 groups.

The Receiver operating characteristic (ROC) curve was used to determine the sensitivity and specificity of syndecan-4 in determining the triple-negative subtype. Data from the same databases, TCGA database, and Metabric.

RESULTS

Metabric analysis for syndecan-4 showed decreased expression in breast tumor patients (n=1,758) compared to control patients (n=140) (Figure 1). Suggesting that there is a decrease in syndecan-4 in breast tumors.

To analyze the relationship of syndecan-4 in the different subtypes, the patients were divided into triple-negative (n=98),

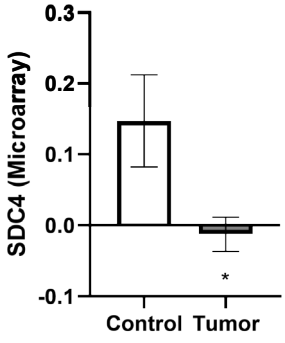

Figure 1: Syndecan-4 gene expression in breast tumor patients compared to control patients. It was analyzed using the Metabric database (Microarray). There were 1758 tumor patients and 140 control patients. A decrease in syndecan-4 gene expression was found in breast tumor patients. The bars represent the means and the lines the standard error. T-test *p<0.05. Breast tumor patients were found to have lower syndecan-4 expression when compared to the control sample.

luminal A/B (n=358), and HER2+ (n=58) (Figure 2). Both RNA-seq (Figure 2B) and microarray data were analyzed in the TCGA database (Figure 2A). There was a decrease in syndecan-4 expression in patients with triple-negative breast tumors when compared to the other subtypes.

Data from the Metabric database was analyzed for confirmation. There were 1140 luminal A/B patients, 220 HER2+ patients, and 398 triple-negative breast tumors. There was a decrease in syndecan-4 expression in patients with triple-negative breast tumors when compared to the other subtypes (Figure 2C).

ROC curve analysis was conducted on both databases. Patients with triple-negative breast tumors were compared with patients with breast tumors with other molecular subtypes. The ROC curve with the TCGA database data had an area under the curve of 0.75 with both the Microarray analysis (Figure 3A) the RNA-seq analysis (Figure 3B) and the ROC curve with the Metabric database data (Figure 3C) had an area under the curve of 0.72; showing that syndecan-4 expression can acceptably differentiate triple-negative breast tumor patients from other subtypes. The results are summarized in Table 1.

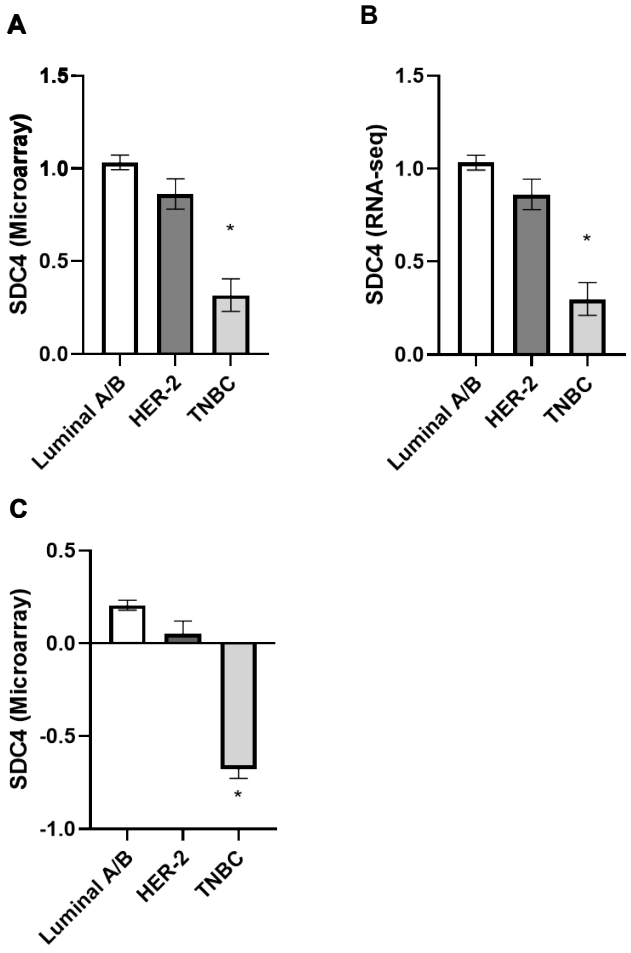

Figure 2: Syndecan-4 gene expression in patients with different breast tumor subtypes. A, Microarray data (TCGA database), TNBC, triple-negative breast tumor (n=98), luminal A/B, luminal A breast tumor and luminal B breast tumor (n=358), HER-2, HER2+ (n=58). B, data obtained from RNA-seq (TCGA database), with TNBC, triple-negative breast tumor (n=98), luminal A/B, luminal A breast tumor, and luminal B breast tumor (n=358), HER-2, HER2+(n=58). C, Microarray data (Metabric), TNBC, triple-negative breast tumor (n=398), luminal A/B, luminal A breast tumor and luminal B breast tumor (n=1140), HER-2, HER2+(n=220). The bars represent the means and the lines the standard error. ANOVA *p<0.05. Both databases showed that patients with triple-negative breast tumors had lower syndecan-4 expression when compared to different subtypes.

DISCUSSION

Syndecan-4 expression is altered in pathological states, knee osteoarthritis and aortic coarctation seem to increase syndecan-4 expression, for example[12,13]. In breast tumors, there is a decrease in syndecan-4 expression when compared to healthy breast tissue.

Syndecan-4 is known to be highly expressed in normal breast tissue, but studies in control patients have shown that there are changes in syndecan-4 expression depending on the phase of

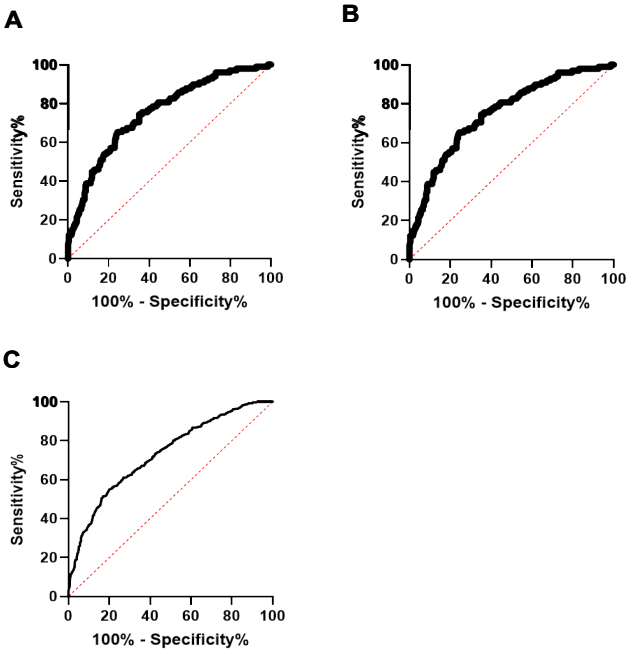

Figure 3: ROC curve of syndecan-4 expression to differentiate patients with triple-negative breast tumor. A, Data obtained from Microarray (TCGA database), where TNBC, triple-negative breast tumor (n=98), luminal A/B, luminal A breast tumor and luminal B breast tumor (n=358), HER-2, HER2+(n=58). B, data obtained from RNA-seq (TCGA database), with TNBC, triple-negative breast tumor (n=98), luminal A/B, luminal A breast tumor, and luminal B breast tumor (n=358), HER-2, HER2+(n=58). C, data obtained from Microarray (Metabric), with TNBC, triple-negative breast tumor (n=398), luminal A/B, luminal A breast tumor, and luminal B breast tumor (n=1140), HER-2, HER2+(n=220). In the TCGA database, both techniques had an ROC curve of 0.75. In the Metabric database, the ROC curve was 0.72. Showing that syndecan-4 expression can differentiate patients with triple-negative breast tumors.

Table 1: Summary table of the results obtained.

Database	Number of patients	Technique	Results	Statiscs
Metabric	1758 breast tumors 140 controls	Microarray	Decreased expression in tumors	T-test p<0.05
Metabric	1140 patients - luminal A/B220 patients - HER2+ 398 triples-negative breast tumors	Microarray	Decreased expression in triple-negative tumors	ANOVA p<0.05
TCGA	358 luminal A/B 58 HER2+ 98 triple-negative	RNA-seq	Decreased expression in triple-negative tumors	ANOVA p<0.05
TCGA	358 luminal A/B 58 HER2+ 98 triple-negative	Microarray	Decreased expression in triple-negative tumors	ANOVA p<0.05
Metabric	1140 patients - luminal A/B 220 patients - HER2+ 398 triples-negative breast tumors	Microarray	ROC curve discriminates the triple-negative subtype from the other molecular subtypes	The area under the curve of 0.72
TCGA	358 luminal A/B 58 HER2+ 98 triple-negative	RNA-seq	ROC curve discriminates the triple-negative subtype from the other molecular subtypes	The area under the curve of 0.75
TCGA	358 luminal A/B 58 HER2+ 98 triple-negative	Microarray	ROC curve discriminates the triple-negative subtype from the other molecular subtypes	The area under the curve of 0.75

the menstrual period[6,14]. Studies using estrogen receptor silencing showed that there was an alteration in syndecan-4 expression. These data suggest that syndecan-4 seems to have its gene expression regulated by steroid hormones[6,14]. Lendorf et al. also showed that syndecan-4 is less expressed in tumors with low or absent expression of estrogen and progesterone receptors[9].

Therefore, the data obtained in the present study showing a decrease in syndecan-4 in triple-negative tumors corroborates the data described in the literature, since this subtype usually shows lower expression of both receptors when compared to other breast tumor subtypes.

It is worth noting that the results on syndecan-4 expression in tumors are still controversial and little is known about the effect of syndecan-4 on tumors since the effect seems to vary with the type of tumor.

Lambert and co-authors demonstrated that syndecan-4 silencing seems to stimulate angiogenesis by increasing $VEGFA_{165}$ signaling, in addition, syndecan-4 silencing increased cell migration by increasing the formation of α-5 integrin fibrillar adhesions[15].

A study by Couchman found that syndecan-4 binds to phosphatidyl-inositol and PKCα, causing their activation and consequent alteration of actin. Cells with absent or decreased syndecan-4 expression show altered cytoskeleton and focal adhesion[16-18].

Syndecan-4 also sequesters and decreases the activity of RhoG (Ras homolog family member G) and Rac1 (Rac Family Small GTPase 1). In the absence of syndecan-4, RhoG and Rac1 are constitutively activated[19]. Both RhoG and Rac1 are related to increased cell migration and the formation of invadiopodia[20,21].

Conclusion

In silico tests show that decreased syndecan-4 expression is related to triple-negative breast tumors. Thus, syndecan-4 could be a potential biomarker since analysis of syndecan-4 expression in breast tissue biopsy could help diagnose the different molecular subtypes since syndecan-4 expression is decreased in triple-negative breast tumors when compared to other molecular subtypes.

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