Frequency distribution of SPARC in triple-negative breast cancer patients

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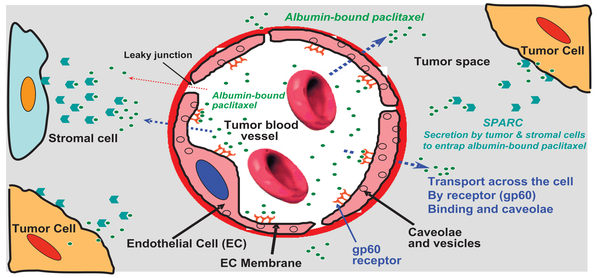
Abstract

Background: SPARC (secreted protein acid rich in cysteine) belongs to a group of extracellular matrix proteins and promotes adhesion of cells to the matrix. As such, expression of this protein plays an important role in tumor development in breast cancer and has a significant bearing on patient prognosis and long term survival. It is also known to predict response to *nab*-paclitaxel in certain tumor types including breast cancer. In 2005, the FDA approved a solvent free formulation of paclitaxel for the treatment of metastatic breast cancer that utilizes albumin bound (nab) technology (Abraxane; *nab*-paclitaxel). Clinical studies have shown that *nab*-paclitaxel is significantly more effective than paclitaxel. Our study was designed to evaluate the frequency distribution of SPARC among breast cancer patients with a special emphasis on triple negative patients in which identification of a novel therapeutic target is warranted.

Methods: We analyzed tumor SPARC expression by immunohistochemistry (IHC) using a monoclonal (R&D Systems) and a polyclonal antibody (Exalpha Biologicals). Immunoreactivity was assessed by scoring the percentage of tumor cells stained in each field and by the intensity of staining. A cutoff point of 2+ and >30% stained tumor cells were considered as positive.

Results: From our analysis of 885 breast cancer patients profiled, a total of 262 patients were triple negative for ER, PR and HER2. Among the triple negative subset, 42% stained positive for SPARC expression. A total of 138 patients were HER2 positive out of which 47% stained positive for SPARC expression. A total of 434 patients were hormone receptor positive out of which 41% stained positive for SPARC expression. Further, there were a total of 318 SPARC positive patients as determined by positive staining by either the monoclonal or polyclonal antibody staining. Fifty out of 318 cases (15%) stained positive by both antibodies and the remaining stained positive by either monoclonal (59%, 185 out of 318) or polyclonal (26%, 83 out of 318) antibody thereby revealing the lack of concordance between the two SPARC antibodies and emphasizing the importance of utilizing both antibodies for detection of SPARC expression.

Conclusion: We conclude that SPARC is over-expressed in a subset of triple negative, HER2 positive and hormone receptor positive breast cancer patients. There was insignificant difference in SPARC expression among these different subsets of breast cancer patients as determined by Fisher's Exact Test. Our study suggests that *nab*-paclitaxel may serve as a therapeutic agent for breast cancer patients that over-express SPARC. The novelty of our study is the SPARC expression data in the triple negative subset of breast cancer patients as it may open up new therapeutic possibilities for these hard to treat cancers. To the best of our knowledge, this is the first study involving a large patient pool in which SPARC has been investigated in a single clinical laboratory using standardized IHC with two different SPARC antibodies.



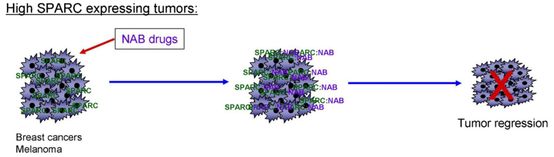
Mechanisms for the transport and accumulation of albumin-bound paclitaxel in tumors. The transport of albumin bound paclitaxel complexes across the endothelial barrier of tumor microvasculature is facilitated by gp60 receptor and caveolin-1 mediated transcytosis. The accumulation of albumin-bound paclitaxel in tumor is enhanced by the presence of albumin binding protein SPARC in the tumor interstitium. Entry of paclitaxel into the cells (tumor or stromal) likely occurs by rapid exchange of albumin bound paclitaxel to the lipidic components of the cell membrane. *Desai N et al Translational Oncology. 2009;2(2);59-64.*

Background

SPARC (Secreted protein acidic and rich in cystein) also known as osteonectin and BM40 is a secreted glycoprotein that has the ability to bind to a number of proteins of the extracellular matrix and by doing so modulates growth factor efficacy, tissue remodeling, angiogenesis and embryonic development. SPARC has been found to be present in high levels in fibrocytes and endothelial cells which are involved in tissue repair.

High SPARC expression is also found in tumor cells when compared with normal background breast tissue. Furthermore, the level of SPARC RNA was found to be significantly higher in ductal tumors when compared with other tumor types. Several studies to date have examined the use of SPARC as a prognostic marker in breast cancer and it has been suggested that a negative association exists between high SPARC levels and overall survival of patients. SPARC levels were also associated with lymph node metastasis. However, contrary results have been reported and the prognostic association of SPARC in breast cancer patients is not clearly defined yet.

The recent use of nanoparticle albumin bound drugs, similar to a *nab*-paclitaxel, approved for use in patients with metastatic breast cancer could be used to exploit the high levels of SPARC in breast tumors. Albumin bound drugs takes advantage of the ability of albumin to transcytose across tumor blood vessel. The benefit of wrapping albumin around an active drug is to eliminate the need for solvents and deliver higher concentrations of tumor targeting chemotherapy without the solvent related toxicities. In the case of high SPARC expressing tumors, the affinity of albumin(and hence albumin bound drugs) to SPARC would allow nab drugs to specifically target these tumors, thereby leading to higher drug accumulation within tumors. The image below taken from a review by Tai IT and Tang MJ. SPARC in cancer biology:Its role in cancer progression and potential for therapy. Drug Resistance Updates. 2008; 11(6);231-246 shows the advantage of using nab drugs including *nab*-paclitaxel in high SPARC expressing tumors. Further, Desai et al 2009 have shown that SPARC over expression may correlate with response to *nab*-paclitaxel in a retrospective analysis of head and neck cancer patients.



Results

Figure 1a: A total of 885 breast tumors were stained with a monoclonal and a polyclonal SPARC antibody to detect the expression of SPARC. As seen in the Venn diagram, 50 out of 318 cases (15%) stained positive by both antibodies and the remaining stained positive by either monoclonal (59%, 185 out of 318) or polyclonal (26%, 83 out of 318) antibody thereby revealing the lack of concordance between the two SPARC antibodies.

Figure 1a

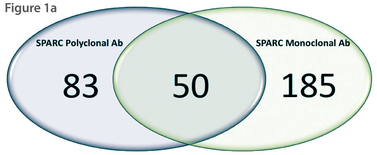


Figure 1b: Lack of concordance in SPARC staining between the monoclonal and polyclonal antibodies. A matched pair showing negative staining by SPARC monoclonal antibody (image on left) and positive staining by SPARC polyclonal antibody (image on right).

Figure 1b

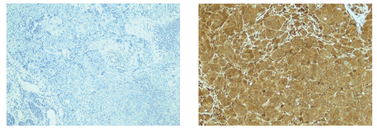


Figure 2

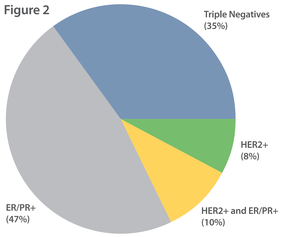


Figure 2: A total of 885 cases were stained with ER(1D5, DAKO), PR (PgR636, DAKO) antibodies to determine the hormonal status. When ER and or PR stained positively, the sample was considered ER/PR positive. Further, HER2 IHC (A0485, DAKO) and FISH was done to determine over expression and amplification of HER2. Samples were considered HER2 positive if HER2 was over expressed and/or amplified. The percentages of triple negative, hormone receptor positive, HER2 positive with and without ER/PR positivity is depicted in the pie chart at left.

Figure 3

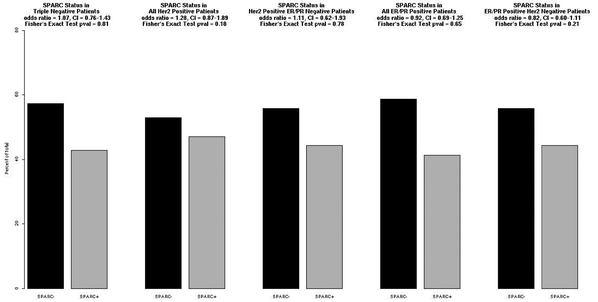


Figure 3: A total of 885 breast cancer patients were stained with ER, PR and HER2 and the patients were subdivided into **a)** Triple negatives (n=262); **b)** HER2 positive (n=138); **c)** HER2 positive, ER/PR negative (n=61); **d)** ER/PR positive (n=434) and **e)** ER/PR positive, HER2 negative (n=357) categories. We then compared SPARC expression in these 5 breast cancer subsets using Fisher's Exact Test. For the purposes of this study staining by either the monoclonal or the polyclonal antibody was considered as positive SPARC staining. All statistical analyses were performed using R statistical software. The p-values for each association along with the odds ratios and their corresponding 95% confidence intervals are shown in the figure above.

Table 1

Table 1: Expression of Secreted Protein Acidic and Rich in Cystein (SPARC) in breast cancer.

Breast Cancer Subtypes	% SPARC positivity
Triple Negative breast cancer	42.7
HER2 positive breast cancer	47
HER2 positive, ER/PR negative breast cancer	44
ER/PR positive breast cancer	41
ER/PR positive, HER2 negative breast cancer	49.5
All Breast cancer	36

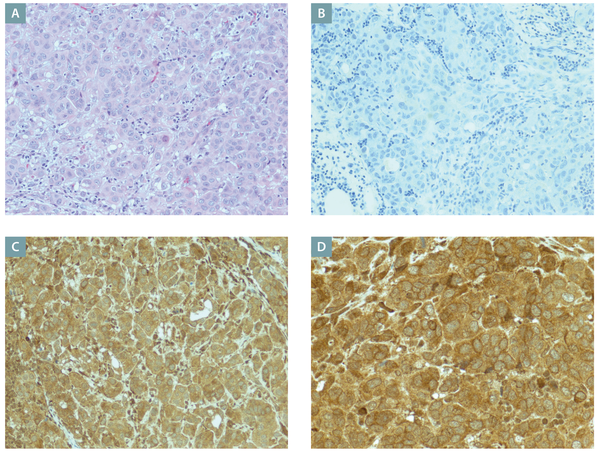


Figure 4: SPARC over expression was analyzed by immunohistochemical staining in breast cancer tumor samples. **A)** H&E; **B)** Negative expression of SPARC; **C)** Positive expression of SPARC (20x); **D)** Positive expression of SPARC (40x)

Conclusions

- This study reveals that there is lack of concordance between the SPARC monoclonal antibody (R&D Systems) and the SPARC polyclonal antibody (Exalpha Biologicals) emphasizing the importance of utilizing both antibodies for the purposes of SPARC profiling for segregating patients with the maximum potential benefit from *nab*-paclitaxel.
- In triple negative breast cancer patients, SPARC protein levels were upregulated in about 42% patients.
- The expression of SPARC in the HER2 positive breast cancer patients was 47% and in the hormone receptor positive group the expression of SPARC was 41%. There was insignificant difference in SPARC expression among the different subsets of breast cancer as determined by Fisher's Exact Test.
- We speculate that the breast cancer patients with high SPARC expression may potentially benefit from *nab*-paclitaxel therapy.

References

1. Tai IT and Tang MJ. SPARC in cancer biology:Its role in cancer progression and potential for therapy. *Drug Resistance Updates.* 2008; 11(6);231-246
2. Desai N et al. SPARC expression correlates with tumor response to albumin-bound paclitaxel in head and neck cancer patients. *Translational Oncology.* 2009;2(2);59-64.
3. Hsiao Y-H et al. SPARC (Osteonectin) in breast tumors of different histologic types and its role in the outcome of invasive ductal carcinoma. *Breast Journal.* 2010;16(3);305-308.