Frequency of TLE3 over-expression in breast carcinoma subtypes including a large cohort of triple negative patients.

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Abstract

Background: The taxanes are an important class of agents for the treatment of a broad range of malignancies including breast cancer. They improve survival in patients with early stage and metastatic breast cancer. Transducin-like enhancer of split 3 (TLE3) is a transcriptional repressor which influences growth and microtubule stability and its expression has been implicated in response to taxane therapy in breast cancer. We investigated the tumor expression of TLE3 in breast cancer patients, including a large cohort of the triple negative subtype.

Methods: We analyzed TLE3 (M-201), ER(1D5), PR(PgR636) and HER2/neu(Polyclonal) expression by immunohistochemistry in 978 breast cancer patients. Immunoreactivity was assessed by scoring the percentage of cells stained in each field and by the intensity of staining.

Results: To sub-classify the 978 breast cancer patients, we utilized hormone receptors (ER and PR) and HER2 expression/amplification. Overall, 36% of the total breast cancer patients were hormone receptor positive, 15% were HER2 positive and 49% were triple negative. The percentage of triple negative patients was higher in our cohort, given the fact that molecular profiling services are used more frequently for this subtype. A total of 477 patients were triple negative of which 61% stained positive for TLE3 expression. Of the 150 HER2 positive patients, 73% stained positive for TLE3 expression as compared with 82% TLE3 positivity in the 351 hormone receptor positive patients. By pairwise comparison, the hormone receptor positive vs triple-negative subtype showed the highest statistical significance in ratios of TLE3 positives ($p = 2.5e\text{-}10$).

Conclusions: Our results show that TLE3 is over-expressed in the majority of HER2 positive and hormone receptor positive breast cancer patients. Interestingly, the frequency of over-expression of TLE3 was lowest in the triple negative subtype thereby making it more important to identify those patients in this group who are most likely to respond to taxanes therapy. To our knowledge, this is the first study providing a comprehensive review of TLE expression in breast cancer subtypes.

Introduction

The transducin-like enhancer of split (TLE) family of proteins are transcriptional co-repressors of a wide variety of transcription factors and play multiple roles in development and tumorigenic pathway. TLE3 is periodically expressed during the M phase of the cell cycle. Since TLE3 proteins are known to interact directly with chromatin and chromatin associated proteins it may identify cells in a differentiation state particularly sensitive to cell cycle perturbation.

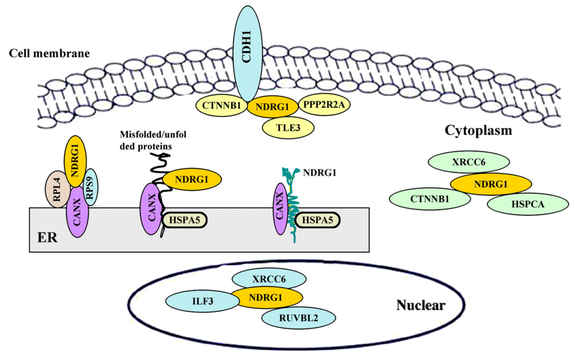
TLE3 interacts with Notch and Wnt pathways and is implicated in the control of epithelial differentiation. Their overexpression is found in several epithelial cancers including breast cancer. They act downstream of the APC and beta catenin by binding to transcriptional complexes. Studies have indicated that Wnt pathway regulates cytoskeletal activity and aberrant Wnt signaling impacts chromosome segregation and spindle orientation.

Based on recent reports, the association of TLE3 with outcome to taxane therapy was apparent in a subset of breast cancer patients receiving adjuvant taxane based therapy as well those who received neoadjuvant taxanes as part of their regimen. These findings have also been noted in ovarian (Samimi et al 2011) and NSCLC (Ross DT et al 2010) patients. While these data are exploratory, expression of TLE3 in breast cancer patients and the possibility of using TLE3 as a tool to predict response to taxanes warrants further study. Even though it is not entirely clear as to how TLE3 predicts taxane response, but it is possible that it acts as an indicator of Wnt pathway activity which impacts cytoskeletal integrity and in turn taxane sensitivity.

Our study evaluated the expression of TLE3 in a large cohort of breast cancer patients to determine the distribution of TLE3 in the hormone receptor positive, HER2 positive and the triple negative subtypes of breast cancer patients.

Since taxanes are substrates for multidrug resistance protein mediated efflux, and their efficacy is compromised in cells that overexpress it, we also evaluated the expression of these biomarkers namely, Pgp and MRP1 in all three breast cancer subtypes.

Classical model of TLE3 acting as a transcription repressor



TLE3 signaling pathway. TLE3 genes are members of the Notch signaling pathway that inhibit transcriptional activation. They do not interact with DNA directly but are recruited to the regulatory region of target genes by DNA binding transcription factors. Figure taken from Tu LC et al. Mol Cell Proteomics 2007; 6; 575-588

Results

Distribution of molecular subtypes of breast cancer

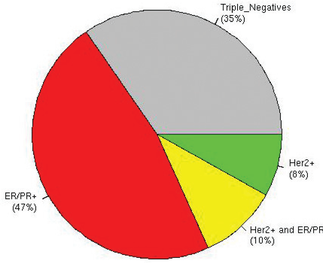


Figure 1: A total of 978 cases were stained with ER(1D5, DAKO), PR (PgR636, DAKO) antibodies to determine the hormonal status. When ER and or PR stained positively, the sample was considered ER/PR positive. Further, HER2 IHC (A0485, DAKO) and FISH was done to determine over-expression and amplification 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 (left).

Images of TLE3 staining in breast tumors

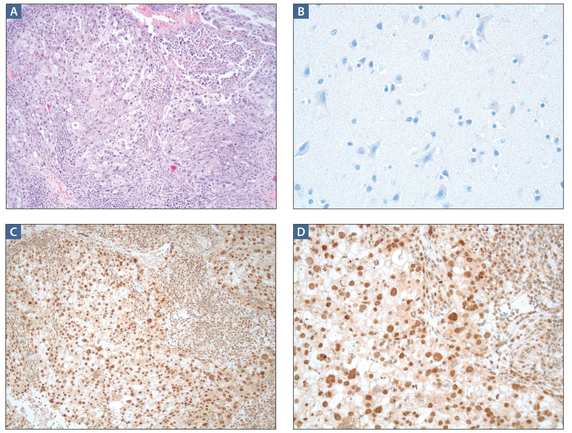


Figure 2: Protein expression of TLE3 was analyzed by immunohistochemical staining in breast cancer tumor samples. A) H&E; B) Negative expression of TLE3; C) Positive expression of TLE3 (20x); D) Positive expression of TLE3 (40x)

Expression of biomarkers predictive of taxane response in breast cancer patients.

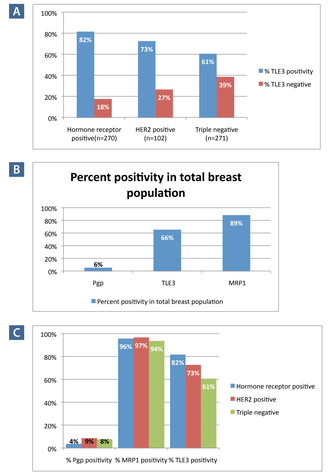


Figure 3A shows the frequency of TLE3 positivity and negativity in hormone receptor positive, HER2 positive and triple negative breast cancer cohort. **Figure 3B** shows the frequency of taxane predictive biomarkers in the total breast cancer population studied. The drug resistance protein, Pgp was present in 6%, TLE3 was present in 66% and MRP1 was present in 89% of the total 978 patients profiled. **Figure 3C** shows the frequency of expression of Pgp, MRP1 and TLE3 in the three different breast cancer subtypes. MRP1 was found to be high in all three subtypes of breast cancer patients. TLE3 was found to be significantly higher in hormone receptor positive patients as compared to the triple negative patients ($p = 2.5e\text{-}10$). Expression of Pgp was found to be below 10% in all three subtypes.

Conclusions

- TLE3 is expressed in majority of HER2 positive and hormone receptor positive breast cancer patients. Among the three subtypes tested, the frequency of TLE3 expression was lowest in the triple negative subtype.
- The expression of drug resistance protein, MRP1 was above 95% and the expression of Pgp was below 10% in all three breast cancer subtypes profiled, highlighting the fact that MRP1 is the predominant multi-drug resistance protein in breast cancer patients.
- Understanding the taxane predictive biomarkers in individual tumors and breast cancer subsets is an important issue with potential clinical and therapeutic impact.

References

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