Extensive searching for breast cancer splicing variants revealed a protein isoform of TRIM37 as a potential biomarker with clinical relevance

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BACKGROUND: Alternative splicing occurs in about 90% of multi-exon human genes, increasing the transcriptomic diversity and proteomic complexity. Splicing variants preferentially expressed in human tumors can be used as potential molecular markers, contributing to more accurate diagnosis and prognosis, and can be also explored as therapeutic targets for more specific treatments.

HYPOTHESIS: Individual exons-containing splicing variants can be reliable candidates for clinical application. The aim of this study was to identify over expressed splicing variants in breast tumors and test them as potential molecular biomarkers.

METHODS: For that, 75 exons previously identified by computational analysis as expressed only in breast tumor tissues and not in their normal counterpart, were comprehensively analyzed. A combined approach of customized exon-arrays and tailored RT-qPCR assays was used to assess breast cancer samples and cell lines, as well as non-neoplastic breast samples and normal cells, to select splicing variants specifically expressed in tumors. For further functional validation, RNA interference (siRNA) technology was used and protein expression was assessed by Western Blot analysis. Proliferation and colony formation assays were performed for assessing possible changes in cells growth.

RESULTS: A total of 28 exons were found as overexpressed in both breast tumor samples and cell lines in comparison to breast normal samples. After comprehensive analysis using stringent criteria, it was possible to select 3 candidates with great potential to be splicing variant biomarkers. In this work we focused in a splicing variant of TRIM37 gene. The *TRIM37*-exon 23 containing variant (TRIM37-E_{23}) was validated as overexpressed in an independent group of tumor breast samples. Moreover, a remarkable increase of TRIM37-E_{23} protein isoform was detected in breast cancer cell lines in comparison to normal breast cell lines, and its expression was preferentially augmented in hormone receptor (HR) positive breast cancer cell lines. Functional assays using MCF7 cells, which express estrogen (ER) and progesterone (PR) receptors, showed increased proliferation and anchorage-independent growth of cells stably expressing TRIM37 in comparison to TRIM37 knockdown (TRIM37^{KD}) cells and controls. Re-expression of TRIM37 into TRIM37^{KD} MCF7 cells showed rescue of proliferation in comparison to controls. Notably, correlation of TRIM37-E_{23} splicing variant expression with clinical and histopathological information of the 59 breast cancer patients investigated in this study showed significant positive associations with the expression of ER ($p<0.009$) and PR ($p<0.024$). Importantly, TRIM37 splicing variant skipping the exon 23 (TRIM37-EΔ_{23}) showed no significant associations with these hormone receptors, indicating that the associations are intrinsically related to the presence of exon 23. This work highlights the importance of individually investigating splicing variants of a specific gene for identifying more precise cancer biomarkers. Additionally, this study reveals TRIM37-E_{23} as a protein isoform with oncogenic potential in luminal breast cancers, and opens new perspectives for the design of more specific targeting therapies for treating HR positive breast cancer patients, in special those that do not benefit from hormone therapy or acquire resistance to it.