Functional interaction between SEPT9_v1 and EGFR in Triple-negative and Inflammatory Breast Cancer

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The poor prognosis for patients with inflammatory breast cancer (IBC) emphasizes the need to better understand the molecular signature of this disease with the goal of developing effective targeted therapeutics. Importantly, 20-40% of IBC cases are triple-negative breast cancers (TNBC) in which targeted hormone therapy is not effective. Interestingly, approximately 1/2 of cases of IBC overexpress the epidermal growth factor receptor (EGFR), a member of the ErbB family of receptors involved in cell proliferation, migration, and cytoskeleton remodeling. Recently, SEPT9, a member of a large family of cytoskeletal proteins important in diverse biological processes, was found to interact with EGFR. Dysregulation of SEPT9 isoforms have been associated in different cancer types, including breast cancer. To date, no studies have looked at the potential pro-oncogenic role of SEPT9 isoforms in IBC or TNBC. We will examine the functional relationship between SEPT9_v1, cytoskeleton remodeling and EGFR signaling in the IBC cell lines SUM149, SUM190 and TNBCs. Our hypothesis is that SEPT9_v1 contributes to oncogenesis by regulating the activity of ErbB receptors in IBC and/or TNBC. To test our hypothesis, generation of stable cell lines with knockdown or overexpression of SEPT9_v1 using lentiviral and delivering vectors will be developed. Establishment of 2D and 3D cultures of the cell lines will be performed to analyze if the acquisition of pro-oncogenic phenotypes and the effects on cytoskeleton dynamics are SEPT9_v1 and/or EGFR dependent. To assess these phenotypes, we will use cell proliferation assays using alamar blue to determine the viability of cells over time, in vitro transwell migration, invasion assays and fluorescent microscopy to determine if there is aberrant morphology or cytoskeleton remodeling. We will determine the specific effect of SEPT9_v1 overexpression and knockdown to the activation of the EGFR signaling. Stability of ErbB receptors upon SEPT9_v1 overexpression or knockdown will be also measured by protein stability assays and by looking directly in the plasma membrane of cells with fluorescent microscopy. Downstream activation of the EGFR pathway will be assessed by Western blot analysis of activated effector proteins. Co-immunoprecipitation analysis will be performed to test if there is a specific interaction between EGFR and SEPT9_v1. This work will provide new insights into the functional relationship between SEPT9_v1 and EGFR and their contribution to the aggressive metastatic phenotype in IBC. Furthermore, it will help us to identify new potential therapeutic targets for the better management of this disease.