EPRS1 Induces Ac4C Modification of mRNAs Encoding Pluripotency Factors in Breast Cancer Cells

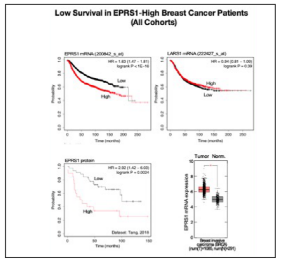
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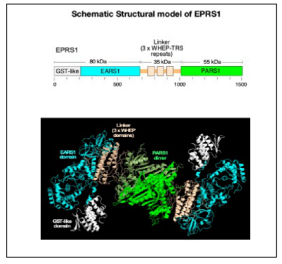
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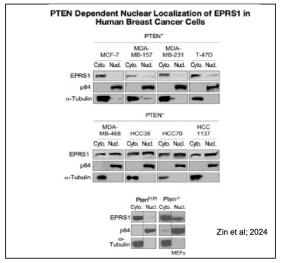
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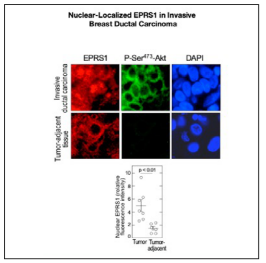
Abstract

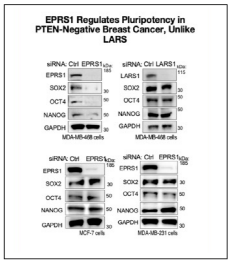
Breast cancer remains a formidable clinical challenge, underscored by the persistence of cancer stem cells (CSCs) that drive tumorigenesis and therapeutic resistance. CSCs are characterized by elevated expression of pluripotency-associated transcription factors, including SOX2, OCT4, and NANOG (SON factors), which orchestrate self-renewal and tumor maintenance programs. Th e downstream transcriptional targets of the SON factors have been investigated in detail; however, the upstream regulation of the factors is less well understood. We previously reported that activation of AKT, or chemical or genetic inhibition of PTEN, induces nuclear translocation of EPRS1 (glutamyl- prolyl tRNA synthetase) a cytoplasmic protein essential for accurate interpretation of the genetic code during translation (1). The contribution of EPRS1 to breast cancer progression is supported by reports that *EPRS1* mRNA and protein are elevated in human breast tumors, and associated with reduced overall survival of breast cancer patients (2, 3). In view of reports that AKT activation is a principal determinant of expression of SOX2 (4) , the master switch of pluripotency factors, we considered the potential role of AKT-induced nuclear localization of EPRS1 in SON factor expression. siRNA-mediated knockdown of EPRS1 in PTEN⁻ MDA-MB-468 and HCC38 cells markedly inhibited expression of the mRNAs encoding the three SON factors, as shown by RT-qPCR; protein expression was similarly diminished. Knockdown of other aminoacyl-tRNA synthetases, e.g., LARS1 (leucyl-tRNA synthetase), was ineffective, thereby showing specificity. Ablation of EPRS1 in breast cancer cells reduced stemness, migration, invasion, and clonogenic potential. Moreover, lim ited dilution assays in mice showed EPRS1 knockdown significantly reduced tumor mass and tumor-initiating cell number. A proteomic analysis of EPRS1-interacting proteins in nuclear lysates of MDA-MB-468 cells gave an insight into the mechanism underlying regulation of SON expression by EPRS1 (1) . Mass spectrometric analysis revealed interaction of nuclear EPRS1 with RNA cytidine acetyltransferase (NAT10), the only enzyme known to acetylate RNAs, including mRNAs, at the 4-cytidine position, i.e., ac ^{4}C modification (5). The interaction was validated by co-immunoprecipitation. Knockdown of NAT10 reduced ac^{4}C modification of SON mRNAs as determined by ac ^{4}C-RIP (RNA immunoprecipitation) as well as SON mRNA expression, indicating an important role for ac^{4}C modification in SON expression. Importantly, EPRS1 knockdown likewise inhibited ac^{4}C modification of SON mRNAs, revealing for the first time a role for EPRS1 in mRNA modification. These results implicate EPRS1 and ac ^{4}C modification of SON factors as key drivers of cancer stem cell activity and suggest new targets for breast cancer treatment.

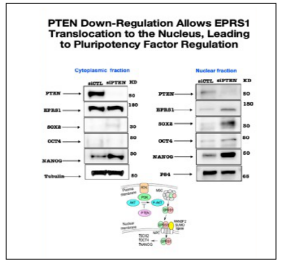


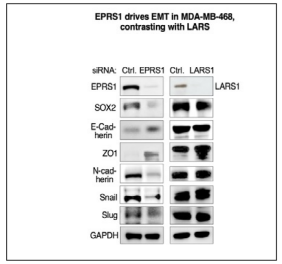


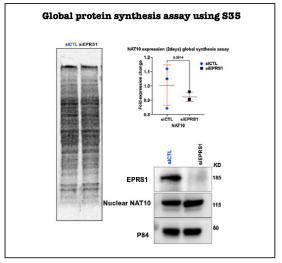


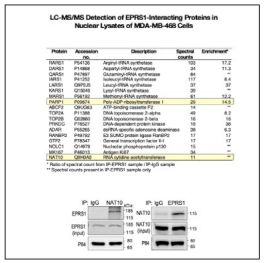


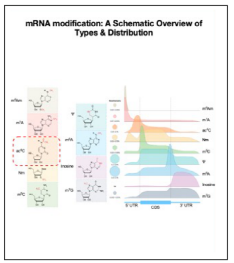


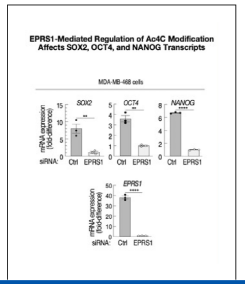


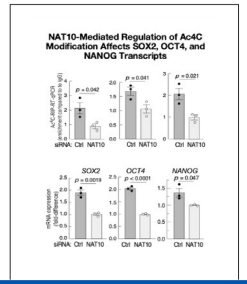


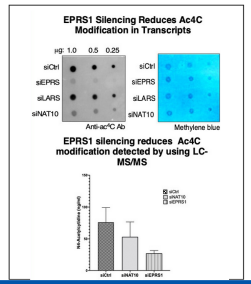


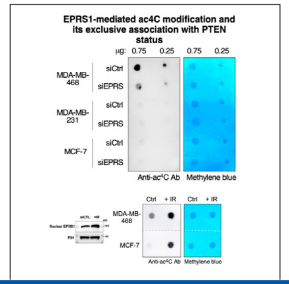


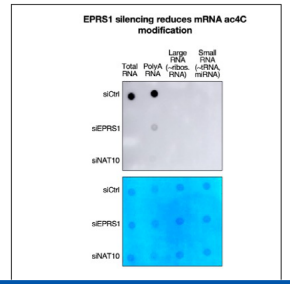


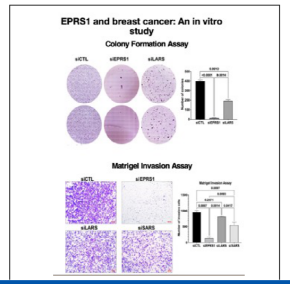


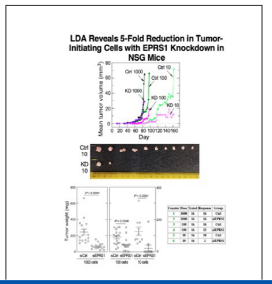


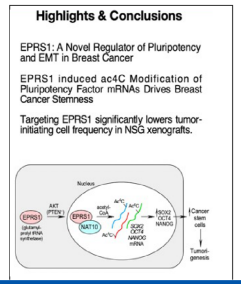












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