Ubiquitination profiling reveals USP7 deubiquitinase as a potential mechanism involved in breast cancer progression

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Introduction: Cancer metastasis is the leading cause of cancer-related deaths. One of the key mechanisms driving metastasis is the epithelial-mesenchymal transition (EMT), characterized by the downregulation of epithelial markers and upregulation of mesenchymal markers. During EMT, crucial proteins such as the transcription factor SNAIL undergo multiple post-translational modifications, including ubiquitination and deubiquitination. Objectives: This study aims to identify targets for ubiquitination during EMT using a combined approach of affinity enrichment of ubiquitinated proteins and proteomics analysis. Materials and methods: The MCF-7 cell line was used as a model to induce EMT through treatment with EGF (10 ng/ml for 48 hours). EMT induction was confirmed by increased SNAIL expression. Cell extracts were subjected to an enrichment strategy for ubiquitinated peptides, utilizing immunoaffinity capture of the C-terminal segment of ubiquitin protein. High-throughput proteomics was employed to identify ubiquitinated peptides derived from proteins altered during EMT induction. Results and discussion: Our analysis revealed 508 ubiquitinated proteins and 723 ubiquitination sites. Among these, 360 proteins had not been previously reported in the PHosphoSitePlus database. Ubiquitinated proteins involved in critical cellular processes were identified, highlighting differences between control and EMT-induced cells. Notably, we identified ubiquitinated USP7, a deubiquitinase known for its role in cancer progression, stabilizing molecules that support angiogenesis and metastasis. Conclusion: The EMT process induces cellular changes associated with protein turnover and localization, affecting numerous molecules within the ubiquitin-proteasome system. Our findings emphasize the role of USP7, a deubiquitinase implicated in the degradation of the p53-MDM2 pathway, as a significant player in EMT. Further investigations into USP7 could elucidate its pro-tumorigenic effects, supporting cancer growth, survival, immune evasion, and resistance to chemotherapy.

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