RESEARCH HIGHLIGHT

Nuclear tetraspanin 8 promotes breast cancer progression

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In a recent work in *Cell Research*, Lu et al. demonstrate that nuclear translocation of the cell surface glycoprotein tetraspanin 8 enhances STAT3 signaling and breast cancer progression. A blocking antibody led to partial tumor control in vitro and in vivo.

Breast cancer is highly heterogenous biologically, morphologically, and medically. Routine assessment includes testing for expression of the estrogen receptor (ER) and progesterone receptor (PR) and for amplification of the gene encoding Human Epidermal Growth Factor Receptor 2 (HER2).[1] Triple Negative Breast Cancer (TNBC) is negative for expression of ER, PR, and HER2, making these tumors resistant to hormonal therapies, which promote differentiation, and HER2 blockade, which drives regression through a variety of mechanisms.[2] Consequently, the prognosis for patients with TNBC is poor, highlighting a need for new therapeutic targets.

The tetraspanin family of integral cell surface glycoproteins help organize microdomains in the cell's plasma membrane. Tetraspanins are also emerging as drivers of cancer.[3] The expression of tetraspanins correlates with tumor type, stage, and outcome. In mouse models, tetraspanins can contribute to tumor initiation, promotion, and progression.[4] Despite their association with disease progression in multiple cancers, mechanistic roles for tetraspanins remain incompletely detailed. Tetraspanin 8 (TSPAN8), in particular, is upregulated in breast cancer stem cells, where it enhances Sonic Hedgehog signaling to promote cancer stemness and contributes to therapeutic resistance.[5]

In a recent work of *Cell Research*, Lu et al. establish a role for nuclear translocation of TSPAN8 in the progression of breast cancer.[6] This builds on the same groups' prior observation identifying TSPAN8 inside the nuclei of multiple cancer cells.[7] In the current study, Lu et al. first establish a positive correlation between expression of nuclear TSPAN8 and aggressive features across multiple cancer types, increased tumor size, lymph node involvement, and distant metastasis. Patients with high expression of TSPAN8 had shorter overall survival compared to those with low expression, correlating nuclear TSPAN8 localization with tumorigenesis.

To clarify whether nuclear TSPAN8 contributed to pathogenesis, the group identified signals that induced TSPAN8 to translocate to the nucleus. EGFR signaling was chosen as a likely candidate because of its established role in tumorigenesis.[8] MCF-7, a human breast cancer cell line, and MDA-MB-231, a human TNBC cell line, were used for immunofluorescence analysis with or without EGF treatment. EGF treatment induced nuclear translocation of TSPAN8, with increased nuclear staining in MDA-MB-231 cells compared to MCF-7 cells. Nuclear fractionation verified that while EGF treatment increased the fraction of TSPAN8 in the nucleus, TSPAN8 translocated from the cytoplasm. Proximity labeling using TurboID confirmed that EGF treatment was not able to induce the plasma membrane extraction of TSPAN8. Signaling mechanisms that induce translocation of TSPAN8 from the plasma membrane to the cytoplasm remain unknown.

To determine how cytoplasmic TSPAN8 entered the nucleus, the group first identified potential binding partners for TSPAN8 using BioID-based mass spectrometry. AKT, a kinase downstream of EGFR, was identified and validated as a binding partner through co-immunoprecipitation. AKT phosphorylated TSPAN8 at Serine129, providing a potential mechanism through which TSPAN8 could be transported to the nucleus by chaperone 14-3-3θ and importin β, proteins previously shown to form a complex with cholesterol-bound TSPAN8 to achieve nuclear translocation.[7] To confirm that phosphorylation was necessary for nuclear translocation, two mutants were generated: $TSPAN8^{S129A}$, a variant that could not be phosphorylated, and $TSPAN^{S129D}$, a variant that mimicked constitutive phosphorylation. Upon EGF treatment, $TSPAN8^{S129A}$ was not detectable in the nuclear compartment and showed impaired binding to 14-3-3θ. In contrast, $TSPAN8^{S129D}$ was detected throughout the cell including the nucleus, and could strongly bind to 14-3-3θ, demonstrating that phosphorylation was required for TSPAN8 nuclear translocation by 14-3-3θ.

BioID also revealed that EGF induced increased interactions between TSPAN8 and the transcription factor STAT3. Interestingly, STAT3 signaling has been implicated in breast cancer progression and malignancy,[9] suggesting this interaction as a mechanism through which TSPAN8 promotes malignant progression. Co-immunoprecipitation confirmed the TSPAN8–STAT3 interaction in MDA-MB-231 cells. Analysis of human primary breast cancers revealed that patients with high levels of both TSPAN8 and STAT3 proteins in the nucleus showed worse prognoses, supporting potential clinical relevance of this interaction. RNA sequencing and ChIP-seq further demonstrated the ability of TSPAN8 to regulate STAT3 target gene transcription, through binding to the promoters of *MYC*, *BCL2*, and *MMP9* upon EGF stimulation. In summary, Lu et al. showed that EGFR signaling activated AKT to phosphorylate TSPAN8, allowing TSPAN8 to bind to 14-3-3θ and importin β for translocation into the nucleus, where it regulated breast cancer progression through promoting transcription of STAT3 target genes.

Earlier in their study, the group found that elevated levels of pTSPAN8 in breast cancer were associated with mesenchymal

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feature, advanced stage, decreased survival, and TNBC subtype. To further explore the potential of TSPAN8 as a therapeutic target, they established an oncogenic role of pTSPAN8 in a xenograft mouse model. Lentiviral vectors containing *TSPAN8*^{WT}, *TSPAN8*^{S129A}, and *TSPAN8*^{S129D} were injected intratumorally into nude mice that had MCF-7 tumors growing in their breast fat pads. Mice injected with *TSPAN8* ^{S129D} grew the largest tumors and displayed the highest levels of the proliferation marker Ki67 in tumor cells, indicating a role for TSPAN8 in promoting cancer progression. The group then generated a human monoclonal antibody against TSPAN8, hT8^{Ab4}, enabling them to test whether blocking nuclear translocation of TSPAN8 could attenuate progression of MCF-10A and MDA-MB-231 cancer cells, as well as TNBC patient-derived organoids. Treatment with hT8^{Ab4} reduced nuclear translocation of TSPAN8 and led to reduced cancer cell growth and cell invasion, particularly MDA-MB-231 cells which were more sensitive to treatment than MCF-10A cells. In MDA-MB-231 tumor xenografts, intratumoral injection of hT8^{Ab4} partially blocked nuclear translocation of TSPAN8 and slowed tumor growth. This treatment did not reduce the size of established tumors. Neither survival, expression levels of STAT3 target genes, nor effects of systemic administration of hT8^{Ab4} were analyzed. Nevertheless, the discovery of a potential mechanism through which TSPAN8 affects breast cancer progression, and the development of a blocking antibody reagent, highlights a potential for blocking nuclear translocation of TSPAN8 as a therapy for TNBC.

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ADDITIONAL INFORMATION

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