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Tumor-specific gain-of-function tGLI1 transcription factor is a novel mediator of breast cancer stem cells and a novel transcriptional activator of cancer stemness genes

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Breast cancer is the second leading cause of cancer-related mortality in women; metastasis to distant organs results in 90% of deaths for these patients. Cancer stem cells (CSCs) are considered the drivers of metastasis. Despite our current knowledge of breast CSCs, there still remains a significant challenge in managing patients with the metastatic breast cancer, underscoring the need for identifying novel regulators of breast CSCs. The hedgehog pathway is an important mediator of stem cells; however, the effect of truncated glioma-associated oncogene homolog 1 (tGLI1), a nuclear effector of the hedgehog pathway and a gain-of-function GLI1 transcription factor, on breast CSCs has never been investigated. Herein, we investigated whether tGLI1 is implicated in breast CSCs by evaluating tGLI1 expression levels in cells grown as monolayer versus mammospheres, as a representation of the stem cell population, and found tGLI1 to be induced in mammosphere culture. Overexpression of tGLI1 promoted mammosphere-forming ability of breast cancer cells, as well as, increased the breast CSC population defined by $CD44^{high}/CD24^{low}$ expression. Further, tGLI1 overexpression transformed normal mammary epithelial cells resulting in increased mammosphere formation and enhanced anchorage-independent growth of immortalized human mammary epithelial HMLE cells. Functional and biochemical assays further showed that tGLI1 promotes breast CSC self-renewal by transcriptional activation of stemness genes including a novel tGLI1 target gene, OCT4, a recently reported tGLI1 target gene (CD44), and known GLI1 target genes (Nanog and SOX2). Bioinformatic analysis of breast cancer patient datasets revealed that activated tGLI1 is associated with shortened time to develop metastasis to the lung, bone, and brain. Furthermore, tGLI1 activation is enriched in HER2-enriched and triple-negative breast cancers, the subtypes with the highest propensity to metastasize, compared to luminal subtypes. Gene Set Enrichment Analysis showed that high tGLI1 activation is enriched in breast cancer with high gene signatures of breast CSCs, radioresistance, and metastasis. We further validated these results by immunohistochemical staining of paired primary breast tumors with lymph node metastases and found that that expression of tGLI1, but not GLI1, was increased in lymph node metastases and that tGLI1 was expressed at higher levels (84-91%) of lymph node-positive metastatic HER2-enriched and triple-negative breast tumors. Lastly, tGLI1 knockdown resulted in decreased mammosphere formation of breast cancer cells and decreased expression of stemness genes, OCT4, CD44, and Nanog. Taken together, these findings establish a novel role that tGLI1 plays in mediating breast CSCs and implicate tGLI1 in facilitating breast cancer metastasis.

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