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Targeting the Achilles' heel of drug-resistant cancer stem cells

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Cancer stem cells (CSCs) have recently emerged as key contributors to drug resistance based on their enhanced abilities to evade apoptosis and survive unfavorable microenvironments. CSCs represent a subpopulation of cells that can self-renew, propagate, reconstitute the tumor heterogeneity, and resist many types of cancer therapies.[1] Determining the specific pathways involved in regulating the development of CSC populations will open doors for novel CSC-targeted therapeutic strategies in malignant cancers.

A collection of cell surface markers that are either specific for the cancer's tissue of origin or expressed by normal stem cells are commonly used for isolation of subpopulations enriched for CSCs.[2] Integrins have long been appreciated as a family of cell surface receptors that are formed by specific combinations of α and β subunits, and certain of these integrins are enriched in CSC populations for various types of cancers.[2] Unlike many other cancer stem markers, the functional role of integrins has been well defined. Specific α/β heterodimer pairs expressed on the surface of a cell serve to "integrate" signals between the extracellular matrix and intracellular signaling pathways in an adhesion-dependent manner, resulting in cell survival, proliferation, and invasion. In addition to this canonical function as an adhesion receptor, integrins can also transmit critical signals in the absence of adhesion or ligation. For example, integrin αvβ3 is unique in its ability to promote cell survival and growth that is independent of adhesion, cell attachment, or matrix ligation. We have demonstrated that integrin αvβ3 in the absence of ligation can drive increased tumor cell anchorage independence and metastasis,[3] which are properties associated with tumor stem cells.

To this end, we recently discovered that integrin αvβ3 expression defines a cancer stem population involved in both acquired and intrinsic resistance to EGFR-targeted tyrosine kinase inhibitors (EGFR TKI).[4] In fact, αvβ3 was both necessary and sufficient to drive stemness and EGFR inhibitor resistance for a range of epithelial cancers, including pancreas, lung, and breast cancer. This finding led us to uncover a novel mechanism wherein integrin αvβ3, in its unligated state, interacts with active (oncogenic or active wild-type) KRAS to promote activation of a RalB–TBK1–NFκB pathway that directs epithelial cancer cells toward a cancer stem phenotype (**Fig. 1**). In fact, forced expression of integrin β3 was sufficient to confer considerable EGFR TKI resistance in lung, pancreas, and breast cancer cells independently of KRAS mutational status, and expression of β3 correlated with erlotinib resistance in lung cancer patients. Whereas CSCs have been linked to a generalized drug resistance and associated with various intracellular mechanisms including an upregulation of drug transporters, we found that integrin β3 selectively drives resistance to receptor tyrosine kinase inhibitors (RTKI) but not standard chemotherapies, highlighting the heterogeneity of CSCs in the tumor.

Although β3-positive cancer cells contribute to a more aggressive, drug-resistant, and stem-like tumor, we have identified an "Achilles' heel" for this subpopulation based on its addiction to the KRAS/RalB/TBK1/NFκB pathway. Through genetic or pharmacological approaches, we demonstrated that targeting this pathway downstream of integrin β3 can reverse the stem-like phenotype and re-sensitize tumors to EGFR TKI. In fact, this can be accomplished using FDA-approved drugs, including the proteosome inhibitor bortezomib, known to inhibit NFκB activation. Not only did bortezomib treatment abrogate the intrinsic EGFR TKI resistance of β3-postive cancers, but when included in a first-line therapy combination with erlotinib, significantly delayed the emergence of acquired resistance in tumor xenograft models, suggesting the potential clinical application of this strategy to overcome EGFR TKI resistance in humans. Our work provides a strong rationale for clinical trials to examine integrin αvβ3 as a biomarker of cancer stem cells and for agents such as bortezomib that can disrupt its downstream signaling pathway.

Whether EGFR TKI treatment selects for an integrin β3-positive resistant population or induces integrin β3 expression through dynamic reprogramming resulting in stem-like properties has yet to be elucidated. However, in some lung cancer patients, erlotinib treatment drives a switch from well-differentiated adenocarcinomas to undifferentiated squamous cell carcinomas, suggesting that EGFR may be a differentiation factor, and inhibition of EGFR may drive integrin β3 expression to induce stem-like properties.

Aside from cancer, αvβ3 has been implicated in adult tissue regeneration and remodeling.5,6 Differential expression of αvβ3 has been reported during angiogenesis, organ development, and wound healing.7 Many angiogenic growth factors and integrins cooperatively activate wild-type KRAS in endothelial cells, and NFκB signaling contributes to endothelial cell

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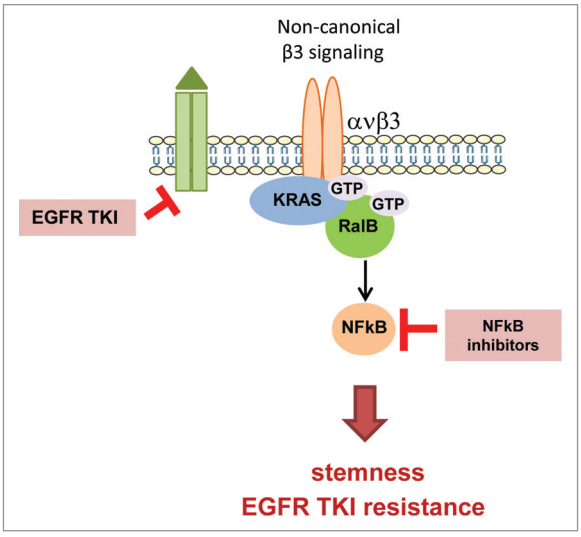

Figure 1. Integrin β3 drives EGFR TKI resistance. Integrin β3 interacts with KRAS to promote RalB activation, resulting in the activation of the NFkB pathway and thereby promoting cell survival. Importantly, ligation of integrin is not required for this signaling cascade. The inhibition of this non-canonical pathway sensitizes β3-positive tumor cells to EGFR TKI. Pharmacologic inhibition of this pathway can reverse cancer stemness and drug resistance.

survival during angiogenesis. Therefore, it is possible that angiogenic endothelial cells utilize a similar β3 integrin-mediated pathway to promote an EMT-like switch that is required for the acquisition of the invasive, sprouting phenotype. In normal adult tissues, the ability of αvβ3 to promote stemlike or mesenchymal properties likely facilitates physiological remodeling and tissue repair. However, when induced on transformed cells, these same consequences of αvβ3 expression trigger a highly aggressive and drug-resistant form of cancer

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