

GD2 and GD3 synthase: novel drug targets for cancer therapy

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Abbreviations and acronyms: CSC, cancer stem cell; EMT, epithelial-mesenchymal transition; FOXC2, forkhead box protein C2; GalNAc, *N*-acetylgalactosamine; GD2, ganglioside GD2 (disialoganglioside); GD3, ganglioside GD3 (disialoganglioside); GD2S, GD2 synthase; GD3S, GD3 synthase; GM3, ganglioside GM3 (monosialodihexosylganglioside); NF- κ B, nuclear factor- κ B; shRNA, short hairpin ribonucleic acid; TGF β , transforming growth factor β .

Our recent study suggests that targeting GD3 synthase (also known as ST8SIA1)—the rate-limiting enzyme in biosynthesis of the breast cancer stem cell marker GD2—abrogates metastasis and depletes the cancer stem cell populations within a tumor, thus providing an effective therapeutic strategy against metastatic breast cancers.

In the last decade or so, it has become increasingly accepted that tumor growth is sustained by a small subpopulation of tumor cells called cancer stem cells (CSCs) that are endowed with self-renewal and tumor-initiating capabilities.¹ The inherent resilience of CSCs is believed to underpin tumor recurrence, therapy resistance, and metastasis with the corollary that the bulk of the tumor is composed of a mixture of proliferating and post-mitotic differentiated cells that have little consequence on tumor sustenance in the longer term. Acceptance of the CSC concept has re-directed therapeutic strategies toward the elusive CSC subset. In addition, it has become clear that targeting solely the tumor bulk may not only fail to eradicate disease but may also inadvertently enrich for therapy-resistant CSCs and lead to tumor recurrence.²

Although the CSC paradigm holds great promise for the development of novel and more effective therapies,¹ the study of CSCs has been hampered not only by the lack of definitive markers but also by the fact that the CSC phenotype is

intrinsically unstable, with tumor cells oscillating between distinct tumor-initiating/CSC and differentiated/non-CSC states.³

We recently showed that the aberrant activation of a latent embryonic program, known as the epithelial-mesenchymal transition (EMT), bestows stem cell properties and confers the migratory and invasive capabilities that underpin metastatic competence.⁴ In addition, we found that cells that have undergone EMT exhibit phenotypic and functional characteristics of mesenchymal stem cells⁵ and present the ganglioside GD2 on the cell surface.⁶ As such, we identified GD2 as a novel breast CSC marker that could be used not only for the isolation of CSCs, but also for their eradication. We demonstrated that inhibition of GD3 synthase (GD3S)—the rate-limiting enzyme for GD2 biosynthesis—using either short hairpin ribonucleic acid (shRNA) or a small molecule known as triptolide reduced the proportion of CSCs and abrogated primary tumor formation.⁶ However, the role of GD3S and GD2 in EMT

and metastasis and, in particular, whether targeting GD3S could alleviate metastasis—the principal cause of cancer-related mortality—remained unclear.

A key finding of the present study by Sarkar et al.⁷ is that GD3S lies at the epicenter of multiple EMT signaling pathways since its inhibition prevents not only the initiation of multiple EMT programs but also the maintenance of the established mesenchymal phenotype. This suggests that targeting GD3S could serve to both inhibit the *de novo* generation of CSCs via EMT and to revert existing CSCs to a more differentiated, therapeutically vulnerable state.

At the molecular level, the transcription factor forkhead box protein C2 (FOXC2), a key downstream effector of several EMT pathways,⁸ transcriptionally regulates GD3S expression, directly linking inflammatory nuclear factor κ B (NF- κ B) signaling to activation of GD3S and the generation of GD2-positive CSCs. This is consistent with the purported roles of inflammation in exacerbating tumor progression. We also found that GD3S

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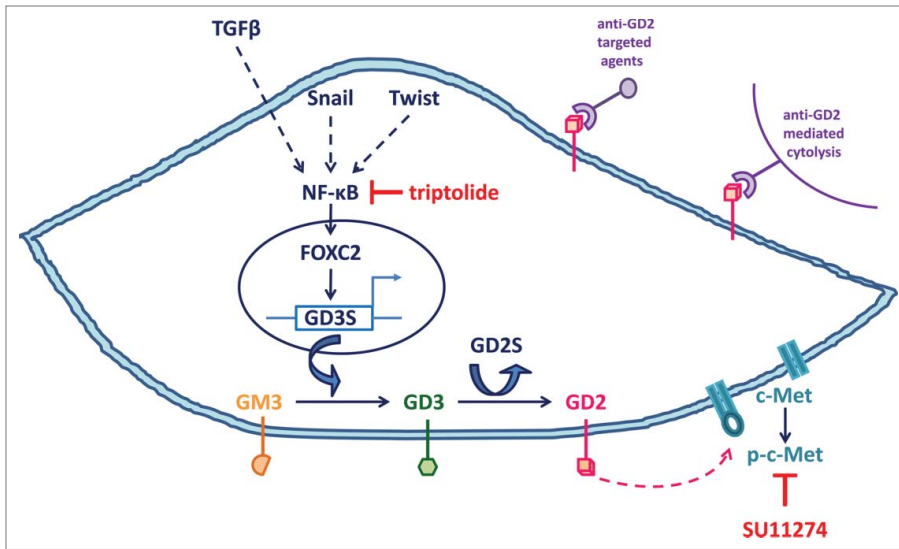


Figure 1. Key players in the GD3 synthase (GD3S)/ganglioside GD2 (GD2)-dependent signaling pathway that regulates stemness, the epithelial-mesenchymal transition (EMT), and metastatic dissemination in cancer cells. Extracellular EMT-inducing signals (e.g., transforming growth factor β [TGF β]) and inflammation elicit the activation of EMT-inducing transcription factors (Snail, Twist) and nuclear factor KB (NF- κ B) that coordinately promote the expression of the central EMT-mediator forkhead box protein C2 (FOXC2), which transcriptionally activates GD3S. GD3S is an enzyme that catalyzes the transfer of sialic acid from cytidine monophosphate-sialic acid to GM3 to produce the ganglioside GD3 (GD3), which is subsequently converted to GD2 via the activity of GD2 synthase (GD2S) and the transfer of *N*-acetylgalactosamine (GalNAc). Although GD2S is directly responsible for GD2 biosynthesis, GD3S is the rate-limiting enzyme for GD2 biosynthesis in cells that have undergone EMT and mesenchymal breast cancer cells. Targeting GD3S and/or *c*-Met signaling using triptolide or SU11274, respectively, reverts the combined EMT/cancer stem cell (CSC) phenotype and abrogates metastasis. Anti-GD2-based immunotherapies may also be used to eradicate CSCs. Broken arrows indicate unknown intermediate steps.

functions via *c*-Met signaling to promote EMT. Whereas GD3S levels correlate with *c*-Met activation in mesenchymal cells, GD3S inhibition elicits a significant decrease in levels of active phospho-*c*-Met and its downstream effector phospho-Akt. Although it remains unclear how engagement of GD2 signaling drives the phosphorylation/activation of *c*-Met, these findings link GD3S/GD2 with key players of a CSC-specific signaling pathway (Fig. 1).

Histological analyses revealed that GD2 is localized at the tumor invasive front that is thought to foster cells actively undergoing EMT,⁹ and that GD3S inhibition markedly reduced invasion of the tumor into the surrounding tissue.

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Accordingly, inhibition of GD3S compromises several *in vitro* properties associated with metastatic competence including migration, invasion, and mammosphere formation, a surrogate assay for the attributes of stem and progenitor cells. Most importantly, genomic and pharmacological inhibition of GD3S compromised metastasis in both experimental and spontaneous metastasis models in the context of an intact immune system. Taken together, these results implicate GD3S in multiple facets of the intrinsic metastatic capabilities of tumor cells.

Although we observed a marked reduction in the incidence of metastasis following GD3S inhibition using shRNA or triptolide, we noted the presence of a

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small number of solitary disseminated tumor cells deposited in the lungs. These findings may reflect incomplete inhibition of GD3S in our tumor models. Nevertheless, these results have high clinical relevance since the expression of high levels of GD3S and phospho-*c*-Met correlate with poor prognosis in triple-negative human breast tumors, a particularly aggressive form of breast cancer with high rates of local and distant relapse.

Exploiting GD2 as a cell surface antigen of breast CSCs not only enables their isolation but also presents a tangible therapeutic opportunity, not least because the GD2 antigen is linked to CSC functionality. The development of high affinity anti-GD2 monoclonal antibodies is already underway, with emerging therapeutic strategies focusing on the use of these antibodies to stimulate an immune response against tumors by triggering monoclonal antibody-mediated cytolysis or to effect GD2-targeted delivery of therapeutic and diagnostic agents.¹⁰ While the effectiveness of immunotherapies based on anti-GD2 antibodies may be somewhat limited by the inability of the antibodies to penetrate solid tumors, our results raise the realistic possibility that small molecules that target GD3S and/or *c*-Met signaling may be used, in combination with standard-of-care therapies targeting the tumor bulk, to curb breast cancer recurrence and metastatic propensity.

Conflict of Interest

TRS, VLB, MA and SAM are inventors of a patent application based on the work described here. NS declares no conflict of interest.

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