TGFβ differentiates pro-stemness from pro-invasive phenotypes during cancer cell EMT

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The epithelial-mesenchymal transition (EMT) fuels embryonic tissue development and adult pathogenesis in the context of fibrosis or cancer metastasis. EMT is induced by transforming growth factor β (TGFβ), and molecularly links to processes of cancer cell stemness and invasiveness. Mesenchymal cells can revert to epithelial cells through the inverse process called mesenchymal-epithelial transition (MET). It remains unclear as to how TGFβ differentiates cancer stemness or invasiveness via EMT. Aiming at identifying contexts that could reveal differences in phenotypic responses to TGFβ, we established a breast cancer fluorescent cell model that is based on its responsiveness to TGFβ. The *E-cadherin* promoter, as a fluorescent marker driver, best phenocopied the majority of EMT and MET features. TGFβ promoted 3D oncosphere formation with low-RFP content, suggesting enrichment of epithelia-mesenchymal cells (partial EMT) in the oncosphere. Under 3D context, autocrine TGFβ supported dynamic generation of non-fluorescent mesenchymal cells that initiated explorative migration in the surrounding space. The autocrine TGFβ action was verified by measuring its secretion extracellularly and by blocking its biological action using the potent TGFβ type I receptor inhibitor LY2157299. In contrast, prolonged exogenous TGFβ stimulation failed to support motility from oncospheres but instead enhanced oncosphere growth. After EMT, TGFβ also induced extracellular vesicle secretion, transporting pro-EMT signals onto recipient cells via a rich transcriptomic population, assessed by sequencing of the vesicular cargo RNAs. Among, these cargo mRNAs, and after cloning 16 of them, we identified novel regulators of TGFβ signaling. Orthotopic mammary transplantation experiments of oncospheres in mice, revealed that prolonged exogenous TGFβ treatment promoted tumor-initiating capacity while it failed to support tumor intravasation and lung metastasis. Thus, pro-stemness or pro-invasive phenotypes by TGFβ are differentiated based on the multicellular architectural context into which the EMT takes place.