ABSTRACT

Spread of a primary tumor to distant organs (termed metastasis) is responsible for >90% of deaths due to cancer. Metastatic breast cancer is typically incurable, necessitating identification of molecular basis of metastatic proclivity. Metastasis suppressor genes (MSGs) have been shown to negatively regulate invasion-metastasis cascade, and provide an opportunity to understand molecular mechanisms of tumor metastasis. This study investigated (a) which of the MSGs described to date (>30 in number) could decisively control metastasis of primary breast tumors, and (b) which signaling pathways were involved in metastasis suppression. Integrative analyses of patient samples, survival parameters, cellular, and in vivo assays, identified non-metastatic 23 H2 (NM23H2/NME2) as a key regulator of breast cancer metastasis. Several lines of observations suggested NM23H2 as a crucial metastasis suppressor: analysis of gene expression datasets from patient tumors showed consistently reduced level of NM23H2. Importantly, immunohistochemical analyses of autologous lymph node metastases demonstrated diminished NM23H2 expression in comparison to primary breast tumors indicating its role in tumor metastasis. In nude mice models, breast cancer cells with reduced level of NM23H2 seeded more metastases to lung compared to cells with enhanced NM23H2 expression. At a molecular level, enhanced expression of NM23H2 caused wide spread changes in gene expression program of breast cancer cells, notably in genes implicated in epithelial to mesenchymal transition (EMT) pathway and transforming growth factor (TGF) beta signaling. Mechanistically, this involved creation of repressive chromatin environment at the promoter of TGF beta receptor II (TGFBRII) through enhanced recruitment of a histone demethylase, LSD1 in response to NM23H2. This subsequently led to diminished crucial phosphorylation events in TGF beta receptor regulated Smad cascade; phosphorylation of SMAD3 was diminished, physical occupancy of E-cadherin promoter by SMAD3/4 repressor complex decreased. Taken together, negative regulation of TGF beta signaling by NM23H2 ensured up-regulation of genes promoting epithelial organization (such as E-cadherin, occluding, CAR among others) and down-regulation of genes imparting mesenchymal characteristics (such as
fibronectin, vimentin, and slug among others). Examination of cellular morphology confirmed that enhanced expression of NM23H2 resulted in gain of epithelial characteristics. In line, NM23H2 expressing cells showed lesser efficiency in executing several steps of invasion-metastasis cascade such as decreased potency to invade through three dimensional extra cellular matrix, and reduced ability to cross through a layer of endothelial cells. These observations suggest inhibition of TGF beta mediated EMT by NM23H2 as a potent mechanism of reducing metastatic seeding during breast cancer progression. It is expected that approaches that increase expression of NM23H2 in primary tumor should decrease the metastatic burden in breast cancer patients.