COX2 regulation of breast cancer bone metastasis

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Abbreviations: COX2, cyclooxygenase 2; DCIS, ductal carcinoma in situ; MDSC, myeloid derived suppressor cell; PGE2, prostaglandin E2; Treg, regulatory T cell

Harnessing the immune system, either by potentiating immune responses or by inhibiting cancer cell-elicited immunosuppressive mechanisms (immuno-editing), to understand and counteract tumor progression is currently at the forefront of cancer research. A subset of CD4+ T cells known as regulatory T cells (Tregs) is instrumental in the maintenance of normal peripheral tolerance and in the control of immune responses to pathogens. Tregs mediate immunosuppressive functions by directly inhibiting T cells, killing them or suppressing clonal expansion. Notably, Tregs can dampen many of the host defenses utilized against cancer, making Treg recruitment by developing tumors a critical step in the evasion of antitumor immune responses. Both pre-clinical and clinical studies have associated the progression of various neoplasms to the high levels of circulating and/or intratumoral Tregs. For instance, in human breast cancer patients, the percentage of Tregs at the tumor site is positively correlated with disease progression to normal tissue to ductal carcinoma in situ (DCIS), and from DCIS to invasive carcinoma. Despite the correlation between Treg accumulation and worsened disease outcome, the mechanisms by which Tregs promote tumor progression remain unclear. Of note, the levels of cyclooxygenase 2 (COX2) and of its main product prostaglandin E2 (PGE2) have also been associated to poor outcome in many tumor models and clinical studies. Although reports have correlated the upregulation of COX2 with increased levels of Tregs in breast cancer, no mechanistic data on this observation was available.

While attempting to elucidate the role of COX2/PGE2 in breast carcinoma progression, we observed that—compared with poorly aggressive mammary TM40D tumor cells—TM40D cells overexpressing COX2 (TM40D-COX2) exhibit an increased rate of bone metastasis, which is comparable to that of a highly-metastatic mammary cancer cell line (TM40D-MB), an effect that can be ablated by the stable depletion of COX2 with short-hairpin RNAs (shRNAs). As these cells did not differ relative to in vitro and in vivo proliferation rates, the effects of COX2 on metastatic potential must reflect proliferation-independent phenomena. Additionally, the overexpression of COX2 in TM40D tumor cells altered the immunological profile of tumors, shifting it from one characterized by high levels of intratumoral CD4+ T helper cells to one featuring intense infiltration by CD4+ FOXP3+ Tregs. Others have shown that PGE2 induces the accumulation of myeloid-derived suppressor cells (MDSCs) and that specific receptor antagonists can block this process. Moreover, 4T1 mammary carcinoma cells inoculated into PGE2 receptor 2 (EP2)-deficient mice grew less efficiently and accumulated lower numbers of MDSCs than similar cells injected into wild-type mice. Although we could not reveal differences in the number of monocytic and granulocytic MDSCs in response to varying levels of COX2 expression/PGE2 production, we cannot rule out that this may influence the activation state of intratumoral MDSCs.

Conversely, our study specifically addressed the ability of mammary tumors developing from cells that express different levels of COX2 to recruit Tregs from the periphery. Purified Tregs that express the PGE2 receptors EP2 and EP4 preferentially migrated in response to factors released by TM40D-COX2 and TM40D-MB cells, an effect that was attenuated using by anti-PGE2 antibodies. Though we suggest one mechanism involving an increased infiltration of the primary tumor by Tregs, others have shown that this phenomenon can be due to the local differentiation of FOXP3+ Tregs from naïve T cells, occurring independent of transforming growth factor ß (TGFß) and interleukin-10 (IL-10). Of note, the PGE2-induced development of Tregs from naïve CD4+ cells requires EP receptors. Specifically, FOXP3 expression in response to PGE2 was significantly
reduced in the absence of EP4 and entirely ablated in the absence of EP2. Although it has previously been shown that PGE$_2$ alone can directly induce FOXP3 expression, we believe that multiple mechanisms can manipulate the immune system to promote an immunosuppressive environment (Fig. 1).

In our attempt to better elucidate the mechanism of PGE$_2$-driven immunosuppression, we observed that COX2-overexpressing tumors contained a higher frequency of apoptotic CD8$^+$ T cells than their wild-type counterparts. These cells are known to be required for the inhibition of tumor progression and metastasis.$^7$ Of note, we observed the accumulation of CD8$^+$/cleaved caspase-3$^+$ cells in specific lymphoid-rich areas of the tumor. Similar observations have been reported by others, including Menetrier-Caux et al., who have recently described the effects of Tregs within lymphoid aggregates in breast tumors.$^9$

Targeting COX2 as the therapeutic approach to breast cancer has been the focus of both clinical and laboratory investigations. A broad inhibition of COX2 may result in undesirable cardiovascular and gastrointestinal side effects that are due, at least in part, to reduced levels not only of PGE$_2$, but also of prostaglandin D$_2$, F$_2$R, I$_2$ (the cardioprotective prostacyclin) and thromboxane A$_2$. Specifically targeting one of the many downstream effectors of COX2 would curb potential side effects for both breast cancer patients and individuals treated with COX2 inhibitors for other indications. Along similar lines, the treatment with anti-EP agents following lumpectomy may result in 2-fold benefits. First, it may prevent the establishment of tumor cells to secondary sites by inhibiting the recruitment of immunosuppressive Tregs, therefore allowing the immune system to clear residual cancer cells. Second, it may decrease osteolytic bone lesions secondary to metastatic dissemination by suppressing the effects of PGE$_2$ on osteoblasts, and hence ultimately inhibiting osteoclast function.$^{10}$ A large amount of data indicates that strategies for the therapeutic targeting of COX2 and Tregs against breast cancer should focus on antagonists that are specific for EP2 and EP4.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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