

Innate immune recognition of breast tumor cells mediates CCL22 secretion favoring Treg recruitment within tumor environment

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Regulatory T cells (Treg) have been reported of poor prognosis for overall survival in primary breast tumors (BT). As CCL22 plays a major role in Treg recruitment within primary BT we deciphered the mechanisms involved in the CCL22 production by breast epithelial tumor cells and propose herein the major role of their innate immune recognition in this production.

Breast cancer is an immunogenic tumor as (1) CD8⁺ T cell response as well as humoral responses against Tumor associated Antigens (TAA) (Her2neu, p53, Muc1) have been demonstrated and (2) CD8⁺ T cell infiltration has been recently reported to correlate with better prognosis.¹ Moreover we recently demonstrated that primary breast tumors (BT) are largely infiltrated by immune cells involved in innate sensing i.e., NK cells, dendritic cells (DC) and macrophages (MΦ) that present an activated phenotype^{2,3} suggesting their stimulation within the BT environment. We also recently reported the presence of strong CD4⁺ T lymphocytes infiltrates in BT⁴ suggesting that all the players required to set-up an efficient anti-tumor response are present within the BT environment.

However, when tumors are clinically detected, this immune response is, in most cases, unable to counteract cancer development because tumors have developed immuno-subversion processes. Several studies including from our group demonstrated the infiltration of BT by immune subsets involved in immune tolerance i.e., plasmacytoid DC³ and CD4⁺CD25^{high}CD127^{neg}FoxP3⁺ regulatory T cells (Treg)^{1,4} and Type 2

MΦ (for review see ref. 5) that are all of poor prognosis for overall survival (OS) in primary BT.

An in-depth *ex vivo* analysis demonstrate that tumor associated Treg (TA-Treg) (1) are activated as they express ICOS, HLA-DR and CTLA-4, (2) are functional as they suppress CD4⁺ T cells proliferation and IFNγ secretion, (3) promote immune-suppressive environment by favoring IL-10 secretion and (4) proliferate *in situ* in contrast to the resting non regulatory CD4⁺ memory T cells and CD8⁺ T cells detected within BT.⁴

In contrast to associated patients' blood Treg, TA-Treg present a selective loss of membrane CCR4, consecutive to an active recruitment through CCL22 secreted within the BT environment. In line with this, BT lacking CCL22 are not infiltrated by TA-Treg independently of their production of CCL17, the other CCR4 ligand. Of note CCL22, but not CCL17 induced the CCR4 downregulation.⁴

CCL22 has been reported to be secreted by myeloid DC, B cells, MΦ, or epithelial cells, all subsets detectable within the BT environment. As previously described for other chemokines (GRO-α/β/γ, CXCL8, MIG, IP-10 and RANTES) we have demonstrated by immuno-histochemistry

(IHC), in peri-tumoral breast tissue, a polarized apical secretion of CCL22 by healthy luminal epithelial cells within lobular acini² (Fig. 1) suggesting CCL22 is part of the mammary gland physiology to control local inflammation associated with menstrual cycle or breastfeeding.

In primary BT context, independently of the molecular subtype of the tumor, CCL22 expression is strongly increased as assessed by IHC but also by ELISA within the BT dilacerations supernatants.

Interestingly, at the systemic level, we could observe a gradual increase in CCL22 plasmatic levels from healthy subjects, patients with primary BT, 1st metastatic relapse or with more advanced BT (Tredan O., manuscript in preparation) that could reflect the tumor burden.

Using BT epithelial cell lines but also primary BT specimens, we recently demonstrated the major role of immune infiltrate in the selective induction of CCL22 but not CCL17 by tumor epithelial cells.² In-depth analyses through *in vitro* experiments using (1) inhibitory antibodies against cytokine receptors and/or cytokines or (2) addition of exogenous recombinant cytokines, demonstrate the preponderant role of a dialog between tumor epithelial cells, infiltrating NK cells

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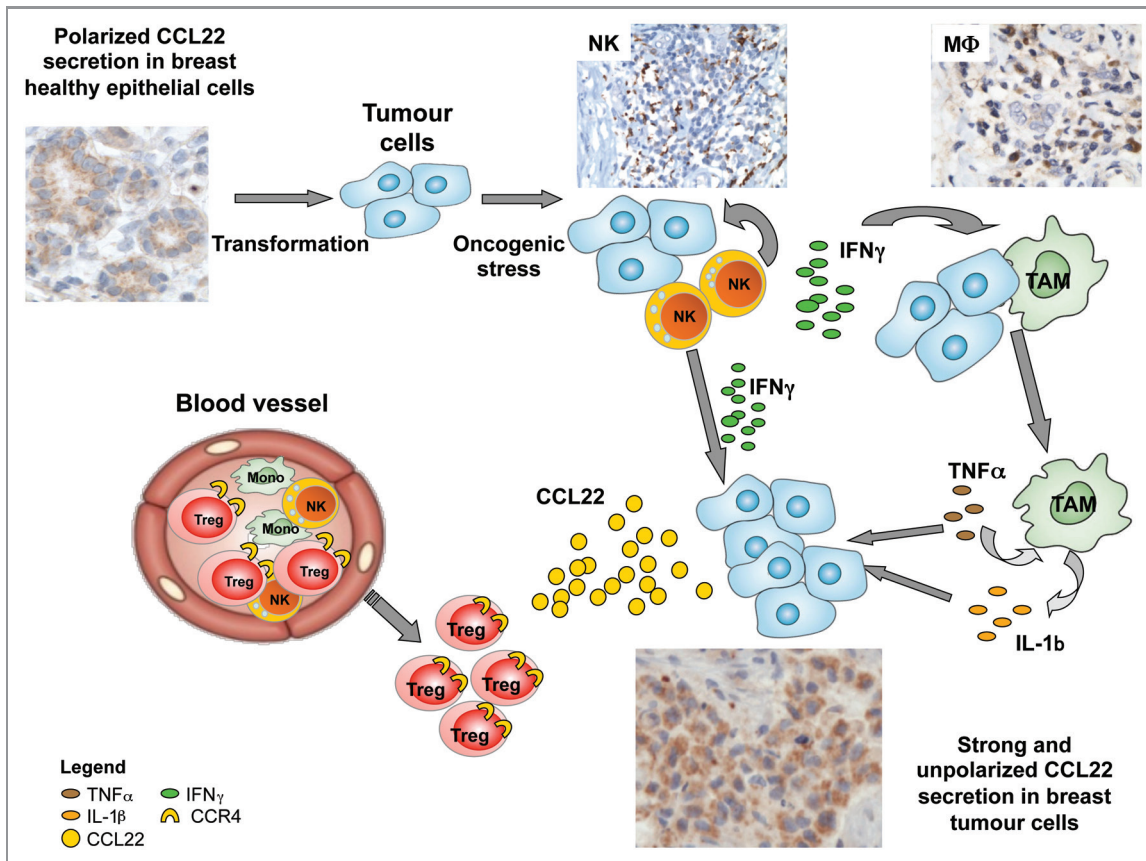


Figure 1. Scheme recapitulating the sequence of events leading to the strong non polarized CCL22 production by tumor cells. Healthy epithelial cells secrete low levels of CCL22 in a polarized manner within the luminal acini, their transformation favor their recognition by infiltrating NK cells leading to IFN γ secretion. IFN γ promoted macrophage activation that will produce TNF α and IL-1 β after interaction with breast epithelial tumor cells. Combined action of IFN γ , IL-1 β and TNF α will induce non polarized strong CCL22 secretion by tumor cells that will induce the recruitment of CCR4⁺ Treg from periphery, leading to CCR4 internalization.

and M Φ for this CCL22 production.² Through these studies we propose the following sequence of events (Fig. 1): (1) NK cells detecting tumor cells secrete IFN γ , (2) IFN γ activates M Φ favoring their secretion of IL-1 β and TNF α , (3) these cytokines act together to increase CCL22 production by epithelial tumor cells. This was further confirmed in ex vivo experiments using primary BT specimens demonstrating the cooperation of M Φ and NK cells to favor CCL22 production by freshly purified tumor cells.

This illustrates a mechanism allowing the breast transformed epithelial cells to counteract the local inflammation involving NK and M Φ to favor Treg recruit-

ment through CCL22 secretion as previously described in chronically inflamed colon.⁶ In turn, TA-Treg may also favor tumor progression via (1) the inhibition of NK cytolytic functions (for review see ref. 7), (2) the conversion, as recently demonstrated in HIV context,⁸ of Type 1 M Φ into Type 2 M Φ that have pro-tumor functions through production of factors promoting angiogenesis, tumor cell proliferation and favoring immunosuppression (for review see ref. 5).

Apart its function in immune cell recruitment, CCL22 has recently been described, in a murine model, to reduce the Ag specific proliferation of CD4⁺ T cells.⁹ In this context, high CCL22

production within the tumor environment may favor the blockade of TAA-specific CD4⁺ T cell proliferation.

All together, these data strongly indicate that CCL22 participate to immunosubversion in BT and will favor disease progression. In this context, CCR4 antagonists have been validated to block in vitro CCL22-mediated recruitment of human Treg and Th2 cells and favored a break of peripheral tolerance to self antigen in murine model by blocking Treg migration.¹⁰ However, selective CCL22 antagonists rather than CCR4 that will not target CCL17 would represent a more selective target to prevent Treg recruitment within tumors.

References

1. Liu F, Lang R, Zhao J, Zhang X, Pringle GA, Fan Y, et al. CD8⁺ cytotoxic T cell and FOXP3⁺ regulatory T cell infiltration in relation to breast cancer survival and molecular subtypes. *Breast Cancer Res Treat* 2011; 130:645-55; PMID:21717105; <http://dx.doi.org/10.1007/s10549-011-1647-3>
2. Faget J, Biota C, Bachelot T, Gobert M, Treilleux I, Goutagny N, et al. Early detection of tumor cells by innate immune cells leads to T(reg) recruitment through CCL22 production by tumor cells. *Cancer Res* 2011; 71:6143-52; PMID:21852386; <http://dx.doi.org/10.1158/0008-5472.CAN-11-0573>
3. Treilleux I, Blay JY, Bendriss-Vermare N, Ray-Coquard I, Bachelot T, Guastalla JP, et al. Dendritic cell infiltration and prognosis of early stage breast cancer. *Clin Cancer Res* 2004; 10:7466-74; PMID:15569976; <http://dx.doi.org/10.1158/1078-0432.CCR-04-0684>
4. Gobert M, Treilleux I, Bendriss-Vermare N, Bachelot T, Goddard-Leon S, Arfi V, et al. Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. *Cancer Res* 2009; 69:2000-9; PMID:19244125; <http://dx.doi.org/10.1158/0008-5472.CAN-08-2360>
5. Mantovani A, Sica A. Macrophages, innate immunity and cancer: balance, tolerance, and diversity. *Curr Opin Immunol* 2010; 22:231-7; PMID:20144856; <http://dx.doi.org/10.1016/j.coi.2010.01.009>
6. Izcue A, Coombes JL, Powrie F. Regulatory lymphocytes and intestinal inflammation. *Annu Rev Immunol* 2009; 27:313-38; PMID:19302043; <http://dx.doi.org/10.1146/annurev.immunol.021908.132657>
7. Ralainirina N, Poli A, Michel T, Poos L, Andrès E, Hentges F, et al. Control of NK cell functions by CD4⁺CD25⁺ regulatory T cells. *J Leukoc Biol* 2007; 81:144-53; PMID:16959895; <http://dx.doi.org/10.1189/jlb.0606409>
8. Huang X, Stone DK, Yu F, Zeng Y, Gendelman HE. Functional proteomic analysis for regulatory T cell surveillance of the HIV-1-infected macrophage. *J Proteome Res* 2010; 9:6759-73; PMID:20954747; <http://dx.doi.org/10.1021/pr1009178>
9. Beaty SR, Rose CE, Jr., Sung SS. Diverse and potent chemokine production by lung CD11b^{high} dendritic cells in homeostasis and in allergic lung inflammation. *J Immunol* 2007; 178:1882-95; PMID:17237439
10. Pere H, Montier Y, Bayry J, Quintin-Colonna F, Merillon N, Dransart E, et al. A CCR4 antagonist combined with vaccines induces antigen-specific CD8⁺ T cells and tumor immunity against self antigens. *Blood* 2011; 118:4853-62; PMID:21908423; <http://dx.doi.org/10.1182/blood-2011-01-329656>