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Transforming growth factor β in breast cancer: another new trick for the old dog

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Strapline (to appear before the title in the ToC)

T cells starving tumours

Running Head: TGFβ blockade in CD4⁺ T cells suppresses tumours

Since its identification in the early 1980s[3], transforming growth factor β (TGFβ) has been characterized as a pleiotropic cytokine regulating a plethora of biological processes in cancer including malignant transformation, tumour cell differentiation and proliferation, angiogenesis, and immune function. In normal tissues and early cancerous lesions, TGFβ typically acts as a tumour suppressor by inducing cell cycle arrest, senescence, or apoptosis. However, it becomes a driving force behind tumour progression at later stages of cancer development[4]. Critically, there is no obvious molecular switch that governs the transition between these two conflicting phases[4]. Moreover, even in established tumours, TGFβ signalling can exert a wide range of pro- and anti-tumour effects.

Despite this complexity, academic investigators and the pharmaceutical industry have made wide-ranging efforts to inhibit TGFβ in cancer. The approaches used include neutralizing antibodies, decoy receptors or "traps", small molecule inhibitors targeting the TGFβ receptor kinases, and RNA interference[4-6]. Such agents have advanced to different stages of clinical development but none have entered clinical practice – either because of limited efficacy or excessive toxicity[6]. It seems increasingly clear that the pleiotropic effects of TGFβ in normal and malignant tissues make it a very challenging clinical target. One way out of the labyrinth might be the differential modulation of TGFβ activity in specific cell types, with the goal of more potently targeting its undesirable effects. To this end, it has become critical to identify cell type-specific functions of TGFβ in cancer.

In 2020, the highest hopes for TGFβ inhibition in cancer are centred around its immunomodulatory potential[6]. TGFβ has numerous well-defined immunosuppressive properties including inhibition of $CD4^+$ helper and $CD8^+$ cytotoxic T cell activity, induction of regulatory T cell differentiation, and 'education' of myeloid cells to foster the myeloid derived suppressor cell phenotype[6]. Given this, TGFβ inhibition might serve as a potent means of reverting an immunosuppressive tumour microenvironment to one that supports immune-mediated clearance of tumour cells.

The two recent papers from the Li lab (Liu *et al.* 2020[1]; Li *et al.* 2020[2]) shed new light on how TGFβ inhibition in immune cells might help to control cancer. Specifically, they report the seminal discovery that conventional $CD4^+$ T cells (and not $CD4^+$ regulatory T

cells or CD8⁺ T cells) are critical mediators on the anti-tumour effects of TGFβ inhibition in breast cancer. Using the MMTV-PyMT transgenic mouse model of mammary carcinoma, the authors demonstrate that the specific inhibition of TGFβ in CD4⁺ T cells (achieved through genetic deletion of the TGFβ receptor II [TGFβRII]) induces dramatic suppression of tumour growth. Strikingly, this effect was *completely independent* of CD8⁺ T cells, suggesting that TGFβ inhibition in CD4⁺ T cells acts through a mechanism unrelated to a T helper cell-mediated promotion of CD8⁺ T cell immunity. So, what is this mechanism?

In their paper, Liu *et al.*[1] provide an unanticipated explanation for why inhibition of TGFβ signalling in CD4⁺ T cells inhibits tumour growth. Specifically, inhibition of TGFβ activity in CD4⁺ T cells leads to their upregulation of a range of Th2 cytokines including interleukin-4 (IL-4). IL-4 is a known inhibitor of angiogenesis[7], and using a range of genetic and pharmacologic approaches, Liu *et al.* demonstrate that this CD4⁺ T cell-derived IL-4 inhibits angiogenesis in a manner that induces hypoxia, tumour cell death, and tumour regression. Interestingly, Th1 cytokines IFNγ played little role in this process.

In an accompanying paper by Li *et al.*[2], the authors went on to develop a prototypic therapeutic agent – called the "4T-Trap" – to leverage their mechanistic findings. 4T-Trap is a bispecific receptor decoy comprising the TGFβ-neutralising TGFβRII extracellular domain fused to ibalizumab, a non-immunosuppressive anti-CD4 antibody, and is designed to potently suppress TGFβ activity in CD4⁺ T cells. In keeping with their earlier findings, Li *et al.* show that 4T-Trap normalises tumour vessels, increases tumour hypoxia, and induces tumour regression. Intriguingly, a non-targeted TGFβ trap did not inhibit Th cell TGFβ signalling in tumour-draining lymph nodes, and did not induce these effects. Overall, this second paper provides further proof-of-concept that CD4⁺ T cell-specific inhibition of TGFβ-signalling impairs angiogenesis, and suggests a possible path for exploiting this in the clinic.

The body of work presented by Liu *et al.* and Li *et al*. constitutes a significant advance in how we understand the immunologic effects of TGFβ inhibition in cancer. Most notably, the discovery that CD4⁺ T cells, rather than the cytotoxic CD8⁺ T cells, are the major immune cell mediators of tumour regression in response to the blockade of the immunosuppressive TGFβ is unexpected. Indeed, these findings bring type II immune

responses onto centre stage – the conventional wisdom is that effective anti-tumour immunity is mediated predominantly *by type I immunity*, with the induction of type II immune responses considered less critical[8]. However, in the paper by Liu *et al*., two key components of type I immunity – $CD8^+$ T cells and IFNγ – are surprisingly dispensable. The unexpected effects of TGFβ inhibition (inhibition of angiogenesis) and insight into the value of type II immune responses in cancer run against the current and are thought provoking. Finally, the development of a putative therapeutic strategy to leverage this biology is very exciting.

Any major advance leaves as many questions as it does answers, and several outstanding issues remain with respect to these papers. (i) This work must be interpreted in the context of previous studies. For example, Rakesh Jain's lab previously demonstrated that TGFβ inhibition induces vascular normalization in mammary carcinomas growing *in athymic mice*, indicating that non-immune mechanisms are also involved in TGFβ inhibition-mediated vascular normalization in tumours[9]. This would be in keeping with the well-characterised effects of TGFβ on vascular maturation, and the relative contribution of $CD4^+$ T cells and non-immune cells to this normalisation is not clear. Furthermore, Muraoka *et al.* previously reported suppression of MMTV-PyMT tumour growth (with no vascular phenotype) using a non-cell type-specific TGFβRII-decoy[10], which is difficult to reconcile with the demonstration by *Li et al.* that a non-targeted TGFβ trap had no impact on tumour growth. (ii) In these papers from the Li lab, TGFβ inhibition in $CD4^+$ T cells normalised vessel structure and function, but did not reduce vessel density. This phenotype, distinct from true vascular pruning, is more typically associated with improved oxygenation of tumours rather than the profound hypoxia demonstrated[11]. (iii) Results are limited to one murine model of mammary carcinoma, and the broader applicability of the findings remains unclear. (iv) When considering 4T-Trap as a potential therapy in the clinic, it will be critical to assess potential toxicities including delayed physiological tissue repair (due to effects on angiogenesis) and skewing of the $CD4^+$ T cell responses that might compromise required type I immunity and provoke autoimmunity.

In summary, the works by Liu *et al.* and Li *et al.* offer intriguing new insights into how type II immunity regulates tumour growth, and suggest a role for $CD4^+$ T cells regulating tumour angiogenesis. We eagerly await more data to determine how generalisable their

findings are, and whether they might even have implications outside of oncology.

CONFLICTS OF INTEREST

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Figure Caption

Figure 1. TGFβ blockade specifically in CD4$^+$ T cells suppresses tumour growth through vascular re-organisation. Treating mammary carcinoma-bearing MMTV-PyMT transgenic mice with 4T-trap, a bispecific molecule comprising a TGFβ decoy receptor fused to the Fc portion of an anti-CD4 antibody, promotes the differentiation of CD4$^+$ helper T cells. IL-4 produced by differentiated CD4$^+$ helper T cells mediates the re-organisation of blood vessels within the tumour microenvironment, evidence by enhanced pericyte coverage, induction of regional hypoxia, and hypoxia-induced tumour cell death.