Purinergic Signalling
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Pancreatic cancer immune evasion mechanisms: the immunosuppressive role of P2RX1-negative neutrophils

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Article summary

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies, mainly due to its higher metastatic potential. An immunosuppressive microenvironment is essential to promote tumour immune evasion mechanisms, leading to tumour growth and metastatisation. In a recent study, Wang et al. [1] demonstrated that neutrophils are a critical immune component of the PDAC liver metastatic tumour microenvironment (TME). Specifically, the authors showed that tumours evade antitumour immunity by accumulating a subset of purinergic receptor (P2RX1)–negative neutrophils. By performing RNA sequencing, cytofluorimetric analysis and metabolic assays, they characterised the immune and metabolic phenotype of P2RX1-negative neutrophils, derived from P2XR1 knockout ($P2xr1^{-/-}$) mice, compared to neutrophils from wild-type mice. The results showed that P2RX1-negative neutrophils have significantly increased levels of immunosuppressive molecules, including programmed death-ligand 1 (PD-L1), and enhanced mitochondrial metabolism, consistent with a N2-like protumoural phenotype. Molecularly, the transcription factor erythroid 2-related factor 2 (Nrf2) is upregulated in P2RX1-deficient neutrophils and associated with PD-L1 expression and metabolic rewiring. Tumour-derived soluble granulocyte-macrophage colony-stimulating factor (GM-CSF) was identified as a driver of Nrf2 over-expression and consequent binding on the PD-L1 promoter in P2RX1-deficient neutrophils. The immunosuppressive phenotype of P2RX1-deficient neutrophils contributed to PD-1-induced CD8+ T cell exhaustion. An anti-PD-1 neutralising antibody was sufficient to inhibit the immunosuppressive effects of P2RX1-deficient neutrophils on OVA-activated OT1 CD8+ T cells.

Overall, this study clearly demonstrated that P2RX1-deficient neutrophils infiltrate PDAC liver metastasis and facilitate the creation of an immunosuppressive microenvironment, thereby amplifying the evasion from antitumour immunity and the colonisation of foreign tissues.

Commentary

A TME is essential for tumour growth, metastatic dissemination and drug resistance. Intercellular crosstalk between tumour and endothelial/stromal and immune cells is driven by multiple receptor-ligand systems, as well as by locally synthesised soluble proteins, including chemokines/cytokines, interleukins, interferons, growth and angiogenic factors [2, 3]. Moreover, a TME is persistently inflamed and hypoxic, facilitating the release by injured, necrotic, apoptotic cells of intracellular molecules that, in turn, are responsible for building a tumour-supportive microenvironment. Among these factors, nucleotides (particularly ATP and NAD), basic elements of all living organisms and well known for their intracellular function in energy metabolism, behave as extracellular danger signals, alerting and recruiting the immune system to possible tissue damage [4]. To prevent protracted reactions that lead to chronic inflammation, homeostasis is rapidly restored through a scavenging circuit operated by nucleotide-catabolizing enzymes that produce the immunosuppressants adenosine and inosine, which can re-enter the cell and reconstitute the nucleotide pool. ATP released in the TME promotes rapid inflammation by binding to excitatory ATP purinergic receptors (i.e. inotropic P2XR and metabotropic P2YR subtypes) that

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amplify T cell receptor (TCR) signalling in lymphocytes and promote inflammasome activation in macrophages, dendritic cells and neutrophils [5].

Usually, a tumour develops immune evasion mechanisms to escape from the control of the immune system by shaping an immunosuppressive TME. PDAC, the fourth leading cause of cancer deaths in the USA, owing in part to its early onset of metastasis, has a desmoplastic and nonimmunogenic TME that lacks cytotoxic functional lymphocyte (CTLs) infiltration [6, 7]. Recent studies showed that liver metastatic PDAC contains exhausted CTLs, suggesting that targeting the immunosuppressive TME could be a promising strategy for treating liver metastasis of PDAC [6, 8]. In the paper by Wang et al. [1], the immunosuppressive TME in PDAC liver metastasis was found to be due mainly to the activity of a subset of neutrophils deficient in the purinergic receptor P2RX1 that accumulated in the presence of PDAC metastasis. Neutrophils are a critical component of the TME, with both antitumoural and protumoural activities. However, in late-stage cancers, this immune population displayed potent antitumour properties [9]. P2RX1-deficient neutrophils infiltrating PDAC liver metastasis facilitate the creation of an immunosuppressive microenvironment, inhibiting CTLs responses [1, 10]. This subset of neutrophils up-modulate expression on their surface of immune escape and N2-like molecules, such as PD-L1, and reprogram their metabolism by switching towards an oxidative-mitochondrial metabolism that supports their immunosuppressive features. The authors, exploiting PDAC metastatic animal models, a knockout mouse for *P2rx1* and molecular and functional assays, revealed that (i) tumour cells secrete increased amounts of GM-CSF; (ii) GM-CSF triggers reactive oxygen species (ROS) release in infiltrating P2RX1-deficient neutrophils, directly up-regulating expression of the transcription factor and stress-sensor Nrf2; (iii) Nrf2, in turns, regulates metabolic rewiring of neutrophils and suppression of pro-inflammatory cytokines, while promoting a significant increase of immunosuppressive molecules. Nrf2 directly binds to the promoter of PD-L1, regulating its transcription and facilitating immune evasion of PDAC in a PD-L1/PD-1-dependent manner.

PDAC is a very aggressive metastatic disease, with only 8% of patients surviving more than 5 years after diagnosis. Thus, a better understanding of the mechanisms underlying the metastatic process in this aggressive cancer is critical to improve treatment and patient survival. Overall, this study greatly improves our comprehension of how PDAC evades antitumour immunity by accumulating in the metastatic sites a subset of P2RX1-negative neutrophils that shape a tumour by promoting an immunosuppressive microenvironment. These results add a piece to the complex puzzle of the regulation of the PDAC metastatisation process and indicate possible novel future therapeutic combinations for targeting critical components of the TME.

Declarations

Conflicts of interest Valentina Audrito declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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