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RESEARCH HIGHLIGHT

Interferon-γ induces cancer cell ferroptosis

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Ferroptosis is an atypical cell death modality involving the oxidative destruction of cellular membranes following the failure of a glutathione-dependent antioxidant system. A recent paper by Wang et al. demonstrates that interferon-γ produced by tumor-infiltrating T cells can kill cancer cells through the induction of ferroptosis.

Cancers only develop if they escape from immunosurveillance, and cancer therapies are successful when they succeed in reinstating the immune control of neoplastic lesions.[1,2] This applies to all modalities of tumor treatments including chemotherapy, radiotherapy, targeted therapy and immunotherapy. Tumor-infiltrating cytotoxic T lymphocytes (CTLs) are of among the most important cell types participating in immunosurveillance. Such $CD8^+$ T cells must produce interferon-γ (IFNγ) to mediate anticancer effects, and knockout of the genes coding for IFNγ (*Ifng*) or the IFNγ receptor (*Ifngr*), as well as neutralization of extracellular IFNγ using suitable antibodies, abolish the efficacy of cancer therapies in many preclinical models.[3–5]

In a recent paper published in *Nature*, Wang et al. elucidate the long-standing enigma on the mechanisms through which IFNγ produced by tumor-infiltrating CTL mediates its anticancer effects.[6] Indeed, IFNγ can induce a non-apoptotic modality of cell death that is called ferroptosis, which results from an iron-catalyzed process of lipid peroxidation initiated through non-enzymatic (Fenton reactions) and enzymatic mechanisms (lipoxygenases) that affect polyunsaturated fatty acids.[7] Ferroptosis can be induced by increasing the intracellular iron pool or by inactivating glutathione peroxidase 4 (GPX4), the intracellular enzyme that protects biomembranes against peroxidation damage. The inhibition of GPX4 can be direct (by a series of pharmacological compounds) or indirect by deprivation of glutathione (GSH) through the depletion of its precursor cysteine, as a result of the inhibition of the cystine/glutamate antiporter system xc^- or the trans-sulfuration pathway. System xc^-, a two-component protein complex that involves a regulatory subunit, solute carrier family 3 member 2 (SLC3A2), and a catalytic subunit, solute carrier family 7 member 11 (SLC7A11), promotes the exchange of extracellular cystine and intracellular glutamate across the plasma membrane. Once taken up by the cell, cystine is reduced to cysteine, which is required for the production of GSH.[7]

Wang et al. demonstrate that IFNγ downregulates both system xc^- subunits (SLC3A2 and SLC7A11) at the transcriptional level, thereby causing depletion of the intracellular GSH pool and triggering ferroptosis in cancer cells.[6] Of note, this finding appears to be clinically relevant because the level of expression of mRNAs coding for SLC3A2 and SLC7A11 correlate with poor prognosis and scarce infiltration of human melanomas by IFNγ-producing CTL. Moreover, the treatment-associated downregulation of SLC3A2 and SLC7A11 expression predicts the success of immunotherapy with the PD-1 blocking antibody nivolumab in melanoma patients. In mouse models, combination treatments involving PD-L1 blockade and cyst(e)inase (an engineered enzyme that depletes both cystine and cysteine) enhances the efficacy of T cell-mediated tumor control, supporting the idea that this mechanism of ferroptosis induction can be harnessed for cancer treatments (Fig. 1).

The seminal work by Wang et al. opens a large panel of perspectives that need to be studied in the future:

Wang et al. demonstrate that immunotherapy with immune checkpoint blockers or transfer of cancer antigen-specific CTL into mice causes tumors to manifest signs of lipid peroxidation.[6] It will be interesting to see whether measurement of redox stress within tumors will allow to monitor or to predict the response of cancer patients under immunotherapy as well. For this, it may be important to develop new protocols for tissue banking that avoid oxidation processes during storage.

Ferroptosis and its regulators including system xc^-, GSH and GPX4 cross-talk to other cell death pathways including apoptosis, necrosis and autophagy.[7,8] Hence, an integrated analysis of the state of the distinct cell death machineries connected to each of these lethal subroutines may yield important insights into the propensity of cancers to respond to immunotherapies. Is the level of expression of ferroptosis regulators only relevant to melanoma prognosis (as shown by Wang et al.) or does this regulatory system, alone or in connection with other cell death pathways, also predict the response of immunotherapy for other cancers?

CTL can use a variety of different mechanisms to induce cancer cell death. These include the delivery of cytotoxic granules through the perforin/granzyme system, the expression/secretion of ligands of death receptors (such as CD95 ligand, tumor necrosis factor, TRAIL) and the production of IFNγ.[9] It will be important to weight the relative importance of each of these cytotoxic mechanisms in a systematic fashion. Moreover, it remains to be determined whether cytokines such as IFNγ might exert effects on other elements of the tumor such as endothelial cells[10] to mediate indirect (e.g., antiangiogenic) anticancer effects.

Beyond these biomarker-related and mechanistic aspects, it will be interesting to determine how immunotherapy can be combined with treatments that enhance the propensity of tumor cells to succumb to ferroptosis. Based on their in vitro tumor-killing effects when combined with IFNγ, Wang et al. suggest

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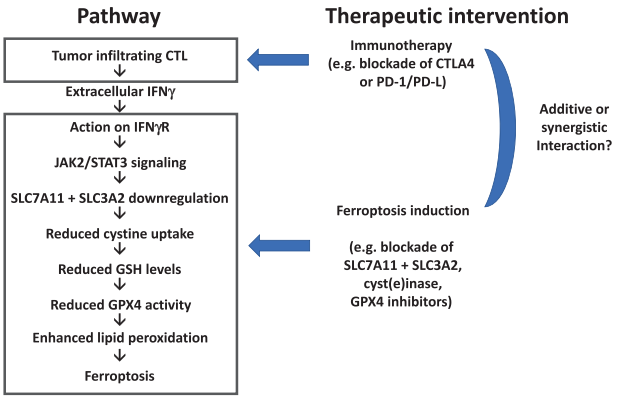

Fig. 1 Schematic overview of the IFNγ-dependent action of CTLs on cancer cells, which results in the induction of ferroptosis

the use of pharmacological GPX4 inhibitors (such as erastin and RSL3) and GSH-depleting agents (such as buthionine sulfoximine, sulfasalazine or cyst(e)inase) for tumor treatment.[6] Among these agents, cyst(e)inase that was administered locally (by intraperitoneal injection) could reduce the growth of transplantable ovarian cancers in mice when given alone, and this effect was further enhanced by simultaneous PD-L1 blockade.[6] It will be important to investigate whether this additive (and perhaps synergistic?) interaction can be confirmed in further preclinical models and clinical trials (Fig. 1). In particular, it will be essential to understand whether cyst(e)inase can be given systemically (for instance by intravenous injection) to mediate anticancer effects. Moreover, it will be interesting to investigate whether pharmacological induction of ferroptosis by targeting GPX4 or system xc^- may be favorably combined with immunotherapy without deleterious side effects.

ADDITIONAL INFORMATION

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REFERENCES

1. Galluzzi, L., Buque, A., Kepp, O., Zitvogel, L. & Kroemer, G. *Cancer Cell* **28**, 690–714 (2015).
2. Fridman, W. H., Zitvogel, L., Sautes-Fridman, C. & Kroemer, G. *Nat. Rev. Clin. Oncol.* **14**, 717–734 (2017).
3. Pitt, J. M. et al. *Immunity* **44**, 1255–1269 (2016).
4. Takeda, K. et al. *Nat. Commun.* **8**, 14607 (2017).
5. Liu, P. et al. *Nat. Commun.* **10**, 1486 (2019).
6. Wang, W. et al. *Nature* **569**, 270–274 (2019).
7. Tang, D., Kang, R., Berghe, T. V., Vandenabeele, P. & Kroemer, G. *Cell Res.* **29**, 347–364 (2019).
8. Song, X. et al. *Curr Biol.* **28**, 2388–2399 (2018). e2385.
9. Voskoboinik, I., Whisstock, J. C. & Trapani, J. A. *Nat. Rev. Immunol.* **15**, 388–400 (2015).
10. Ruegg, C. et al. *Nat. Med.* **4**, 408–414 (1998).