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COMMENTARY

Sometimes simple is not best: The complex γδ T cell interaction with tumour needs depth

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γδ T cells have the potential to be an extremely valuable tool in cancer immunotherapy. They demonstrate cytotoxicity against a number of tumour cell lines, are able to respond rapidly due to their non-MHC dependent activation and are instrumental in recruiting the adaptive immune response. In 2015, Gentles et al. found that tumour infiltrating γδ T cells were the cell type most indicative of a favourable cancer outcome.[1] Many studies have generated data allowing us to understand why γδ T cells are associated with regression and how they can be of significant benefit in a clinical setting. Despite the activity in two-dimensional (2D) in vitro cell culture models, clinical studies show mixed results. This is likely due to the oversimplification of the relationship between effector γδ T cells and tumours. In an effort to overcome this, three-dimensional (3D) tumour models, spheroids and organoids, have been developed. Recently Ou et al. have utilised 3D models with a range of complexity, allowing a greater understanding of changes in γδ T cell activity in a tumour-like environment, and the logical application of strategies to restore tumour killing.[2]

Both Vδ1 and Vδ2 γδ T cells have MHC-independent anti-tumour activity.[3] Malignant cells often have high intracellular levels of small phosphoantigens due to a dysregulation of the mevalonate pathway. These small phosphoantigens bind to the intracellular region of the ubiquitously expressed Butyrophylin 3A1 molecule causing conformational changes in the extracellular domain, allowing for the interaction and activation of the Vδ2 T cell receptor.[4] Phosphoantigens can be increased therapeutically by aminobisphosphonate drugs, such as zoledronic acid. Additionally, γδ T cells display natural killer (NK) receptors, like NKG2D, which can activate the cell through interaction with stress-induced ligands displayed by tumour cells. When activated, anti-tumour responses seen within in vitro 2D models include the expression of death receptors (TRAIL and FASL), the production of cytotoxic molecules like granzymes and perforin, as well as pro-inflammatory cytokines, like IFN-γ.[3]

Clinical approaches utilising γδ T cells include in vivo activation of γδ T cells with aminobisphosphonates and IL-2, or the adoptive transfer of ex vivo expanded γδ T cells. Due to the MHC-independent nature of the γδ T cell interaction, allogeneic effector cells are equally as efficient at tumour killing as autologous cells. This raises the possibility for the manufacture and storage of γδ T cells from healthy blood donors to be used instead of autologous cells, which may be difficult to generate in time from a cancer patient. Results from small clinical studies utilising either in vivo activation or adoptive transfer have, however, so far been disappointing. Enhanced Vδ2 proliferation and activity in diverse tumours, including renal cell carcinoma, gastric cancer, and lung cancer, have been shown, with no severe toxicity associated with the treatment.[5] Few patients, however, show clinical outcomes and even less show complete responses, as reviewed recently by Nussbaumer et al.[5] These variable results highlight the distance between in vitro 2D cell culture and the in vivo reality.

2D in vitro cell culture models are a powerful tool for pre-clinical research, they are economical, simple, and allow for rapid drug and toxicity studies. However, they lack the complexity that comes with cellular interactions and the milieu in the tumour microenvironment. In other systems mouse models are commonly used to address this complexity, providing valuable information on in vivo cancer biology, drug toxicity, and treatment efficacy. One major block to in vivo experimentation is the fact that γδ T cells are dissimilar between mice and humans, and only primates have Vδ2 T cells. Although non-human primates (NHPs) are an appropriate model for γδ T cell studies, there is a lack of available non-human primate tumour models. Alongside the obvious ethical concerns around inducing tumours in NHP studies, the high costs involved with this model are prohibitive. Patient-derived xenograft mouse models, involving the transfer of human tumours to immunodeficient mice, are highly manipulated and also typically depend upon the mouse immune system. Therefore, safety and efficacy cannot be extrapolated to humans.

Tumour spheroids are 3D cell models formed by culturing single-cell suspensions under non-adherent conditions, eventually leading to the spontaneous aggregation of 3D spheres. Spheroids grown in extracellular matrix (ECM) from tumour cell lines are able to mimic micrometastasis or tumour emboli, as well as the ECM organisation found in tumours. Recently, Mizuguchi et al. have shown that spheroids were grown in ECM display basal-apical polarity.[6] Other groups have observed complex internal architecture with lumen formation [7]. The unique characteristic of tumour spheroids is their structure: they have an internal layer of necrotic cells, a middle layer of quiescent cells, and an outer layer of proliferating cells. As outlined recently by Gunti et al., this structure gives resistance to radiation and drugs, mirroring human tumours.[8] Organoids are mini in vivo-like organs, resembling the tumour from which they were derived. Many studies have demonstrated the potential use of spheroid and organoid technology to study immunotherapies, autologous T cell expansion, and assessment of patient responses to anti-cancer drugs and immune checkpoint inhibition. Votanopoulos et al. found that the response of patient-derived organoids to immune checkpoint inhibitors correlated strongly with patient clinical responses.[9] Another advantage of organoids is that they can be cryopreserved, and therefore organoid biobanks are being established as a tool for cancer research. Moreover, tumour organoids can be created using a combination of tumour and stromal cells that may further add to their semblance to in vivo tumours.

Many groups are now using 3D models to examine γδ T anti-tumour responses, broadening the immunotherapeutic possibilities in pre-clinical cancer research. Nozaki et al. demonstrated γδ T expansion with intestinal epithelial organoids and successfully tracked their migratory phenotype.[10] Zumwalde et al. showed that Vδ2 T cells are able to target and kill bisphosphonate treated breast organoids.[11] Ou et al. utilised four separate 3D models of increasing complexity to study γδ T cell anti-melanoma activity.[2] Comparing γδ T cell infiltration, cytotoxicity and checkpoint expression between the separate models allowed them to understand how growing complexity impacted the phenotype and activity of the cells. In particular, they demonstrated an exhausted γδ phenotype, and that immune checkpoint inhibition could restore infiltration and killing.[2]

The lack of timely and economic pre-clinical platforms that are complex enough to mimic human malignancies is a major barrier to developing anti-cancer drugs and immunotherapies. However, 3D culture systems are able to bridge some of the gaps in complexity and, in the case of γδ T cells, the issue of non-relevant animal models. Utilising this organoid approach for γδ T cell immunotherapies can allow for rapid investigations of key checkpoint molecules as well as high-throughput screening of drugs that may enhance their infiltration and anti-tumour activity. The use of organoid cultures will allow the thorough investigation of the complex relationship between solid tumours and an exciting, emerging immunotherapy using a, so far, underutilised tumouricidal cell type, the γδ T cell.

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