EXCLUSION OF T CELLS DURING IMMUNE EVASION AND LACK OF RESPONSE TO IMMUNOTHERAPY BY THE CANCER: CELL TYPES, TRANSCRIPTIONAL PROGRAMMES AND BIOMECHANICS

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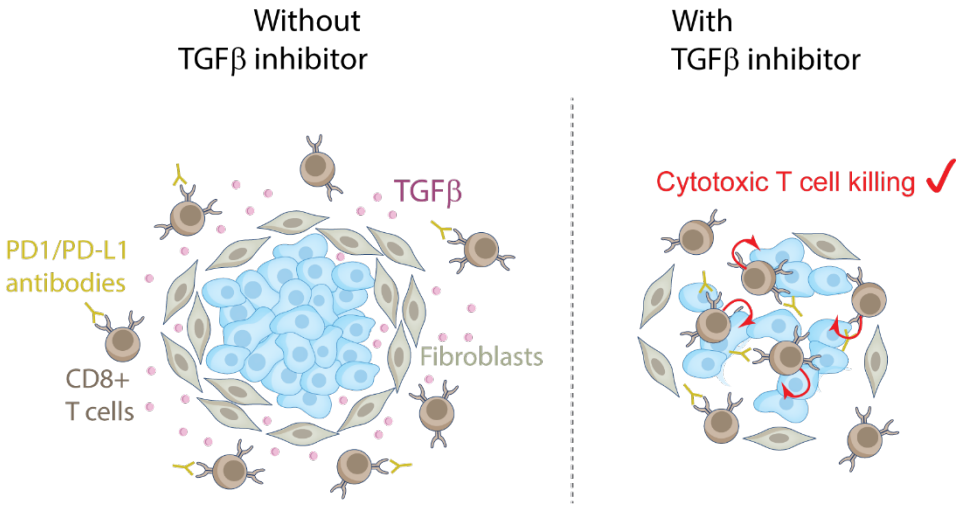
1. Summary

Cancer takes 9.6 million lives each year (GloboCan). Among the most promising therapeutic strategies to treat late-stage cancers are immunotherapies, particularly immune checkpoint inhibitors (ICIs; e.g. antibodies against PD1/PD-L1). However, many common tumour types, including colorectal cancer, inhibit the capacity of the immune system to eliminate the disease by impeding the migration of immune cells into the tumour. These tumours are known as immune-excluded and do not respond well to current immunotherapies. We had discovered that these effects are driven, at least partially, by TGF-β signalling in the tumour microenvironment. TGF-β inhibition promoted strong anti-tumour immune responses and potentiated the efficacy of ICIs. Similar results have been obtained using preclinical models of other types of cancer suggesting that TGF-β inhibitory therapies may be effective for many other types of malignancies that thrive in a rich TGF-β microenvironment, in which immunotherapies have shown little response.



With this project, we intended to characterize the biological basis of T-cell exclusion using models for metastatic colorectal cancer (CRC) that faithfully reproduce the human disease. We investigated whether immune-excluded tumours may reflect a specific chemokine state, the presence of particular barriers, or specific stromal-based inhibition, including forces and repulsion cues that impede T cell infiltration and tumour killing. We rationalized that understanding these processes will not only contribute to increase the efficacy of immunotherapies but also inspire new therapeutic strategies.

The project was carried out as a close collaborative effort of a multidisciplinary team with expertise in CRC biology (Eduard Batlle, IRB Barcelona), single-cell RNA sequencing (scRNAseq; Holger Heyn, CNAG) and biomechanics (Xavier Trepat; IBEC). We sequenced at single cell level tumours generated by MTOs under TGF-beta inhibition, immunotherapy, or both. We characterized in detail the changes upon treatment in the different subpopulations of tumour lymphocytes, myeloid cells, and cancer associated fibroblasts (CAFs). In parallel, we studied how different tumour cells respond to the forces imposed under various conditions mimicking those imposed by the tumour environment, and the dynamics of metastasis growth in a novel pre-clinical model of CRC relapse.

2. Results

We found significant changes in specific populations of immune cells in response to TGF-beta inhibition, with an ECM component playing a major role in TGF-beta-mediated immune evasion. In addition, TGF-beta specified cancer associated fibroblasts (CAFs) with a myofibrotic phenotype (myCAFs). myCAFs exert traction forces that encapsulate tumour organoids causing transcriptional changes in the tumour cells. Altogether these results may translate into novel treatment options for metastatic cancer based on targeting the tumour microenvironment.

We studied the dynamics of metastasis evolution, including the characterization of the tumour microenvironment of early and late metastases, in a novel human-like model of residual disease. This experimental model represents a milestone for fundamental and preclinical research on advanced CRC. We discovered that early metastases are immune infiltrated, and that neoadjuvant immunotherapy prevented disease relapse. These results have important clinical implications, as they suggest that for patients with stage II or IIICRC who do not have metastases at the time of diagnosis (yet, a percentage of them have residual disease and will relapse), neoadjuvant treatment with immune checkpoint inhibitors (ICIs) may prevent the appearance of metastases. Importantly, these results apply to patients with mismatch proficient (pMMR) CRC, which currently are thought not to be responsive to ICIs, opening a novel strategy for their treatment.

We have also identified disseminated occult cells and a candidate cell of origin for metastases in primary tumours. We are convinced that this finding will have profound implications both for the research and clinical communities opening novel lines of research both towards increasing our knowledge about these cells, as well as towards eradicating them. Our preliminary data suggests that these cells disseminate by collective cell migration. Our studies on biomechanics indicate that collective cell dynamics within patient-derived organoids strongly depend on their fate, which might in turn affect T-cell kinetics and their ability to kill cancer cells. We found that different cancer cell subpopulations show mechanical features that promote different steps in the metastatic cascade. Cells negative for the marker LGR5 display a mechanically dynamic but fragile phenotype suitable for invasion, whereas LGR5 positive cells display a stable and resilient phenotype suitable for extravasation and metastatic growth.

The project has also led to the development of a lab-on-chip device that mimics the tumour ecosystem including cancer associated fibroblasts, patient-derived organoids and immune cells. In future studies we plan to use this device as a platform for drug testing and explore its potential for personalized medicine.

3. Relevance and Potential Future Implications

A major breakthrough of this research has been to provide proof of concept that neoadjuvant immunotherapy can eliminate residual metastatic cells and prevent disease relapse post-surgery. This paradigm shift has profound implications for cancer research and oncology, with immediate clinical translation. Preliminary clinical trials are underway, reinforcing the potential of this approach to transform patient outcomes. Moreover, this project has revealed further details on TGF-beta's role in modulating the tumour microenvironment to promote immune evasion in CRC. By delineating its effects on immune cell populations, we have identified novel potential therapeutic targets to disrupt immune evasion and enhance treatment efficacy. These insights may open new avenues for targeted interventions aimed at overcoming resistance to immunotherapies.

Finally, our establishment of human-like mouse models of CRC relapse, and a lab-on-chip device that mimics the tumour ecosystem including Cancer Associated Fibroblasts, Patient-Derived Organoids and immune cells, provide invaluable preclinical platforms for studying disease progression and evaluating therapeutic interventions, ultimately shaping the future landscape of CRC treatment.

4. Publications

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