ImmunoTools *special* Award 2022

Ana Manuela Borges, PhD-student

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Dissect the tumor microenvironment to battle cancer radioresistance and immune escape

According to emergent epidemiological data, breast cancer is the most diagnosed worldwide and the leading cause of cancer-related death in women. The evidence that its incidence and mortality is worsening leads to the urgent need of more efficient therapeutic strategies. This challenge is particularly relevant in triple-negative breast cancer (TNBC), which lacks the expression of therapeutic targets, like estrogen, progesterone and human epidermal growth factor type 2 receptors. Additionally, being considered more radioresistant than other breast cancer subtypes, TNBC is significantly associated with a higher risk of locoregional recurrence following radiotherapy (RT). This points the mandatory need to unveil mechanisms underlying TNBC radioresistance, envisaging the disclosure of novel targets for development of radiosensitive immunomodulatory therapies, improving this poor prognosis disease management.

To address this objective, attention must be paid to other elements of the tumor microenvironment, as immune cells, highly recruited to tumors upon ionizing radiation, modulating cancer cell activities and therapy response. Recently, our group demonstrated that macrophages may differently orchestrate cancer cell response to RT. While irradiated macrophages induced apoptosis in radiosensitive cancer cells, macrophages protected radioresistant cancer cells from death, inhibiting apoptosis (*AT Pinto et al, PLoS One, 2016*). Additionally, using an immunocompetent orthotopic murine TNBC model, immunomodulatory nanoparticles administered in combination with RT potentiated its effect, reducing tumor burden, by modulation of local and systemic myeloid and lymphoid populations (*F Castro et al, Acta Biomaterialia, 2017; F Castro, Biomaterials, 2020*). Therefore, our main goal is to unravel the role of immune cells, in particular macrophages and T cells, on TNBC radioresistance, aiming at identifying novel targets for immunomodulatory therapies.

To address this objective, we will establish and characterize biomimetic 3D-immune-spheroids as a model to study TNBC radioresistance and drug screening. These spheroids combine radiosensitive (MDA-MB-231) or radioresistant (MDA-MD-231-RR) TNBC cells with T cells and macrophages, isolated from healthy blood donors (Centro Hospitalar Universitário São João), using protocols already established at our laboratory. Then, one week-old, radiosensitive or radioresistant-immune-spheroids will be submitted to fractioned RT (5x2.67Gy), mimicking one week of cancer patients treatment. After spheroids dissociation and cell sorting, we will evaluate the impact of RT on cancer cell viability (live/dead staining), apoptosis (Annexin V), proliferation (Ki67) and immunogenicity profile (CD279, PD-L1, CD47, HLA-ABC) by flow cytometry. Using the same strategy, the impact of RT on macrophage (CD14, CD86, HLA-DR, CD163, CD206, CD40) and T cells (CD3, CD4, CD8, CD25, CD69, FoxP3, CTLA4, CD279, Lag3, IFN-γ) profile and activation will be studied. The differences found between radiosensitive and radioresistant-immune-spheroids will be appointed as targets for pharmacological intervention, and the molecular mechanisms explored for novel immunomodulatory therapies to be combined with RT, reverting TNBC radioresistance.

The ImmunoTools collection of flow cytometry antibodies and ELISA sets selected will be fundamental to evaluate the impact of RT on cancer cells viability, proliferation and immunogenicity, on macrophage viability and polarization, and on T cells profile and activation. With your support, we will dissect the role of immune cells on cancer cell radioresistance and immune escape mechanisms, and identify targets for pharmacological intervention, bringing insights on novel immunomodulatory therapies to be combined with RT to revert TNBC radioresistance.

GESINAS - ImmunoTools Award application:
For the past 6 years, I was engaged in several social projects, including VO.U, an Association of Volunteering of University of Porto, in which I made weekly visits to elderly with social and financial needs. In the same association, I was part of a project to protect the environment, helping to renew the forests that suffered from the fires in Portugal and acting on public awareness for nature protection. Also, during my bachelor, I worked volunteering for the association “Rabo de Peixe Sabe Sonhar” with the objective to help the children of Açores that lack of basic needs as education.

GESINAS - **ImmunoTools** AWARD for **Ana Manuela Borges** includes 19 reagents

FITC - conjugated anti-human CD3; CD4; CD40; CD47; HLA-DR; Annexin V-FITC

PE - conjugated anti-human CD4; CD8; CD25; CD40; CD86; CD279; HLA-ABC; IFN-gamma

PerCP - conjugated anti-human CD4; CD8

APC - conjugated anti-human CD3; CD14; CD69

DETAILS more AWARDS