Plano de trabalhos para tese de Mestrado 2018/2019

Exploiting 3D cell models to study the role of macrophages in anaplastic thyroid carcinoma aggressiveness

Supervisors: Catarina Brito; Branca Maria Limón Cavaco (anabrito@ibet.pt; bcavaco@ipolisboa.min-saude.pt)

Lab/Institution: Unidade de Tecnologia de Células Animais - Laboratório de Modelos Celulares Avançados, ITQB-NOVA/iBET. Molecular Endocrinology Group/ Unidade de Investigação em Patobiologia Molecular (UIPM), Instituto Português de Oncologia de Lisboa de Francisco Gentil, Lisboa.

Background:

Thyroid cancer has been showing an increasing incidence in the last years. IPOLFG is the reference center for this malignancy in the South and islands of Portugal, whereby ~ 600 new cases/year are followed in the Endocrine Service.

Anaplastic thyroid cancer (ATC) explosive loco-regional enlargement and invasiveness makes it generally unresectable and resistant to chemo/radiotherapy, therefore, this tumour is one of the most lethal malignancies, with a median survival of 3–4 months. Hence, novel therapeutic strategies for ATC are required.

ATCs are characterized by an intense macrophage infiltration (comprising approximately 50% of the tumour volume). Tumour-associated macrophages (TAMs) form a dense interconnected network with ATC cells and blood vessels. Several studies proposed that TAMs can contribute to the lack of response to therapies and have an important role in tumour progression. Preliminary data from our group suggest that macrophages may have anti-tumoural or pro-tumoural activities. As such, the development of 2D and advanced 3D ATC in vitro cell models, based on cancer cell lines and primary immune cells, are needed to investigate TAMs role in ATC. Furthermore, modulation of the immune microenvironment of ATC may be a novel and potentially effective strategy to control ATC progression and improve its response to therapy.

Project Description

The main aim of this project is to clarify the role of macrophages in ATC aggressiveness and the mechanisms by which, in this tumour context, macrophages may have anti-tumoural or pro-tumoural activities. The specific objectives are to establish and compare 2D and 3D cancer cell models, using representative human ATC cell lines that are available in the Molecular Endocrinology group, and to applying a 3D culture strategy, recently developed by Advanced Cell Models Lab, incorporating tumour and immune components (Rebelo and Pinto et al., 2018).

2D and 3D co-cultures of ATC cell lines and THP-1-derived macrophages or primary human macrophages will be developed. For 3D cultures, stirred-tank culture systems will be used for co-culture of tumour cell spheroids and immune cells, entrapped in alginate microcapsules (Rebelo and Pinto et al., 2018: Estrada et al., 2016). 2D and 3D co-cultures will be employed to investigate the reciprocal effects of ATC cells and macrophages.

Specifically, to the project is divided in 4 tasks:

1) Investigate the effect of ATC cells on macrophage phenotype (M1 or M2), through the analysis of macrophage specific markers and cytokine profiling (flow cytometry, immunohistochemistry, Western blot and cytokine arrays qRT-PCR), employing 3D co-cultures to generate ATC cell spheroids with macrophage infiltration.

2) Investigate the molecular mechanisms involved in the effect of macrophages in ATC cell lines (e.g. expression of factors associated with EMT; migration and invasion capacity) by proteomic analysis, immunocytochemistry, Western blot, cell-based assays.

3) Assess novel pro/anti-tumoural macrophage markers in a series of ATC cases by immunochemistry. These results will be correlated with clinicopathological data.

4) Pharmacologically modulate macrophages in order to improve ATC response to therapy [e.g. CSF-1R inhibitors (BL7945) and stimulation with IL-10 and INF-α].

Timeline

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Task 4										
Thesis										