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Design and characterization of 3d cultures in oropharyngeal squamous cell carcinoma.

Oropharyngeal Squamous Cell Carcinoma (OPSCC) is one of the most important types of head and neck cancer, which is the sixth most frequent type of cancer worldwide nowadays. It is very heterogeneous, as it affects very different areas within the head and neck region, and on top of that, in the last few years scientists in the field have reported the presence of a group of cancer cells that resemble stem cells and difficult the treatment process. For this reason, it is important to identify these cancer stem cells and separate them from healthy stem cells.

This project focuses on studying the expression profile of markers present in these stem cells using different head and neck cell lines, flow cytometry and organoid culture, which are tiny versions of organs obtained *in vitro* from stem cells. For this, we compared the changes in the expression of these cells when they were grown in a 2D culture and when grown in a 3D culture (the organoids) using an artificial extracellular matrix known as Matrigel. In order to do that, we used four markers that are said to induce tumour formation in OPSCC: CD44, NGFR, Lgr5 and MUC1.

This experiment showed that after 3D growth the cells did not recover their initial heterogeneity, but they appear to be similar to undifferentiated tissue. This conclusion was reached by the decrease observed in the expression of MUC1, a marker that is typical of differentiated cells, and by the up-regulation of CD44, which is in turn a marker of stem cells and is tightly linked to NGFR. This means that the spheroids grow as if they were tissue, developing a very similar structure and organization: MUC1 expression decreases as we approach the basal layers, where the CD44 and NGFR-expressing cells are. Lgr5, however, did not show the expected expression, as it was down-regulated in spheroids. This could be due to a defective or incomplete culture medium, a problem that could be solved by adding the adequate supplements that would allow us to obtain tissue-like structures with the correct Lgr5 structure.

We also measured the diameter of the organoids to check which combination of markers was present in the most aggressive tumours, and we observed that those organoids which express CD44 and NGFR had the biggest diameter. This was expected, as the combination of these two markers has been reported to be the most tumorigenic one. These CD44+/NGFR+ cells regain stemness, which makes them a good candidate to detect cancer stem cells in OPSCC.

All this would can be used to confirm the differentiation patterns of tissue and the structure of the organoids, which could be cultured with other types of cells in order to study which kind of interactions take place in the environment surrounding the tumour.