ALDH and ALK, two stem cell-associated genes involved in NB tumour initiation and progression?

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Neuroblastoma (NB) is a typical paediatric embryonal cancer, originating from neural crest-derived sympathetic progenitors, a highly heterogeneous and multipotent cell population. Our interest in this disease lies in its malignant and extremely complex behaviour, as it exhibits a wide range of differentiated phenotypes, from undifferentiated tumours, to tumours containing a neural crest-derived differentiated cell range. The considerable heterogeneity of the disease is mirrored by the range of clinical outcomes, which extends from spontaneous regression to extreme malignancy. The clinical and biological heterogeneity of NB is strongly believed to reflect its tissue of origin.

Heterogeneity within cancer cell populations is common. There are several models regarding heterogeneity in cancer cells. In the "cancer stem cell" model, tumours display a functional cellular hierarchy for malignancy, and are exclusively sustained by a minor sub-population of tumour-initiating cells (TICs) harbouring stem cell characteristics such as self-renewal, drug-resistance, unlimited growth potential. In the "clonal evolution" model, heterogeneity arises from clonal evolution within the tumour, where all cells can potentially drive tumour growth.

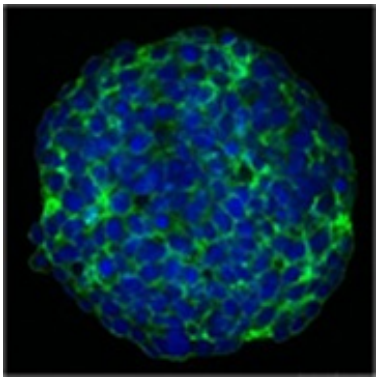

Figure 1: *Typical sphere generated from highly aggressive neuroblasts. Nuclei are stained in blue (DAPI) and cytoplasm in green (phalloidin).*

Considering its particularities, NB qualifies as a pertinent model to explore functional cellular hierarchy and to identify the tumour initiating cells responsible for the development and propagation of the most malignant forms of the disease. Identifying a specific TIC population has strong clinical implications, in particular in the development of targeted therapeutic strategies.

One perennial problem in this field is the absence of specific markers which could be used to isolate TICs. To overcome this hurdle, we took advantage of the *sphere assay* (also known as self-renewal assay) which allows exclusively stem cell-like cells to grow as spheres, while eliminating committed or differentiated cells. By serial passages of spheres, generated from NB metastatic cells, we selected a population of sphere-forming cells and identified its specific gene expression profile (S-profile). Several genes in the S-profile were found to be common to neural crest and neural stem cells, (*CD133*, *MDR1*, *EDNRB*, ***ALDH1A2***, ***PTN***, *GPR177*, *NOTCH3*, *LGR5* and *ABCA1*). Overexpression of these genes was indeed detected in NB tumours, and was found to be associated to their malignant character, thus confirming a putative role of these genes in tumour initiation and progression.

In this project, we focused our investigations on 2 selected genes from the S-profile:

1. Aldehyde dehydrogenase (ALDH)

ALDH include a family of enzymes involved in detoxification of aldehydes and shown to be potentially implicated in stem cell biology as well as in cancers.

High ALDH activity was detected in most NB cell lines. ALDH expression in patient samples was also

shown to increase however in a restricted number of cells during the serial passages of spheres, suggesting that a pre-existing ALDH-expressing cell subpopulation was selected during the self-renewal process. In addition, inhibition of ALDH activity in NB cells, resulted in significant reduction of stem-cell properties, such as self-renewal capacity, proliferation, clonogenic potential and drug-resistance, thus further implicating ALDH in tumour initiation.

2. PTN/ALK

The interest in the tyrosine-kinase receptor ALK, has considerably grown, when it was shown that ALK was overexpressed in a majority (92%) of NB and mutated in 80% of familial and in 8-10% of sporadic NB. In addition, activating ALK mutations were very recently shown to be instrumental in NB oncogenesis, thus qualifying these genes as essential players in tumour initiation. As the putative ALK ligand PTN was included in the S-profile, we explored the impact of wild-type (wt) and mutated ALK expression in NB tumour initiation and progression.

In this aim, murine neural crest stem cell lines (JOMA1), stably expressing the ALK-wt (JOMA-1-ALKwt), or two mutant sequences F1174L, and R1275Q (JOMA1-ALK-F1174L and -R1275Q) were created. We compared the tumour initiation capacities of the different JOMA1 transfectants after implantation in the nude mice adrenal gland, the natural site for NB development. JOMA1-ALK-F1174L and to a lesser extent, -ALK-R1275Q and -ALK-wt cells were shown to initiate extremely rapid and large tumours in animals. No tumours could be generated in the absence of ALK expression in JOMA1 cells, confirming that ALK-wt and both activating ALK-F1174L and ALK-R1275Q mutants were capable of initiating aggressive tumours from normal neural crest stem cells.

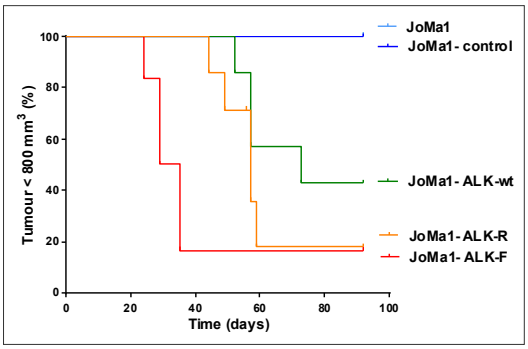

Figure 2 : Survival curves of mice implanted with JoMa1 ALK-expressing cells and controls. Evidence of increased tumorigenic properties of JoMa1-ALK-F1174L, -ALK-R1275Q and - ALK-wt cells as compared to controls

These findings represent the first demonstration of an oncogenic activity of both ALK-wt and ALK-R1275Q mutation, in addition to the ALK-F1174L mutant. They also strengthen the hypothesis of an essential involvement of the wild-type and mutated ALK gene in driving NB development as well as in the later steps of tumour progression.