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In vitro studies of the microenvironmental factors responsible of the proliferation and aggressiveness of desmoid tumor cells

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Desmoid-type fibromatosis (DF) is a rare connective tissue disease characterized by the alteration of the Wnt pathway with the abnormal nuclear translocation of beta-catenin and GSK-3beta. This pathology has a wide variability in growth rate, localization and aggressiveness and it is often correlated to previous local trauma and an altered inflammatory response. This suggests that desmoid tumours require a favourable microenvironment to develop and grow. The hypothesis is that aberrant activation of the canonical Wnt pathway can be associated with inflammatory factors, growth factors, and hormones that promote the development and aggressiveness of the desmoid tumour.

It is known that the deregulation of the inflammatory process can lead to aberrant fibroblast activation and accumulation of ECM (Extracellular matrix) proteins with subsequent tissue fibrosis that can evolve in fibrotic disease and tumours. Desmoid-type fibromatosis (DFs) can be an example of a pathology arising from direct Wnt/ β-catenin signaling alteration (Wnt mediator mutations) as well as indirect Wnt deregulation through the involvement of the microenvironment. We focused our attention on the inflammatory and growth factors studying their interaction with the Wnt/β-catenin pathway.

For this purpose primary cells derived from desmoid tumor (DF) and control cell (Ctr) samples were incubated with different cytokines, growth factors and hormones. Their effect on the growth rate and aggressiveness were evaluated at cellular and molecular levels. The results of this study demonstrated that TGF-β (Tumor Growth Factor-beta) is the compound that shows a pro-proliferative effect and that drives the myofibroblastic characteristics on DF cells.

It is known that Tumor Growth Factor-beta is implicated in tissue repair and in fibrosis process and it is a mediator of the epithelial-myofibroblast transition (EMyT). As alpha-Smooth Muscle Actin is the gene target of the myogenic program we evaluated if Tumor Growth Factor-beta induces the expression of alpha-Smooth Muscle Actin in DF cells. We observed a fibrillar staining in DF cells that markedly increased when treated with Tumor Growth Factor-beta suggesting that Tumor Growth Factor-beta induces the differentiation of DF fibroblast-like cells in myofibroblast cells.

Literature data described a central role of Smad3 in epithelial-mesenchymal tissue transition and fibrosis induced by Tumor Growth Factor-beta. In order to investigate specific interactions between components of the TGF-β and Wnt/ β-catenin signaling pathways, we examined the potential role of Smad3 in Tumor Growth Factor-beta dependent induction of alpha-Smooth Muscle Actin. Ctr and DF primary cells treated with a specific inhibitor of Smad3 phosphorylation (SIS3) demonstrated that the expression of alpha-Smooth Muscle Actin by Tumor Growth Factor-beta is Smad-dependent. The phosphorylation of Smad3 is crucial for Tumor Growth Factor-beta dependent DF cell proliferation and its effect is attenuated by SIS3 in a dose-dependent manner.

On the basis of the results we created an Ampliseq RNA custom panel for the next generation sequencing-based gene expression profiling (Ion Torrent PGM, Life technology), in order to investigate expression of genes that could be related to the different behaviour of DF cells compared to the control cells, with or without addition of Tumor Growth Factor-beta.

We focused our attention on genes connected with Tumor Growth Factor-beta pathway, WNT pathway, and genes involved in interactions between those pathways that are crucial to many biological processes such as the myogenic program. We found several genes differentially expressed in DF cells compared to Ctr samples observing a functional relation between TGF-β and Wnt pathway in the fibroblast differentiation in DF samples. In particular we noticed an overexpression of most of the selected Wnt pathway genes in DF samples. Intriguingly several genes involved in the myogenic program showed a low expression in DF cells compared to Ctr sample cells (SNAIL1 and SNAIL2), while others showed different expression level between DF sample cells (ACTA2 and DES). After Tumor Growth Factor-beta treatment, in Ctr cell samples, we observed a downregulation of most of the Tumor Growth Factor-beta pathway effectors with concomitant upregulation of the inhibitors. In particular the data suggested that Tumor Growth Factor-beta treatment of Ctr cells affected the studied pathways in order to control the proliferative stimulus and the myogenic differentiation process in order to limit its effect.

After treatment, DF cell samples showed gene expression opposite variation compared to Ctr cell samples for several genes, such as ACTA2, SRF, COL1A1, MMP2, and CTGF.

The results of this study highlight that TGF-β plays an important role in proliferation and myogenic feature of DF cells in vitro suggesting a crosstalk between Tumor Growth Factor-beta and Wnt pathway.

Although, per se, these data are insufficient to prove the underlying mechanism, they give an indication of the path to follow in order to unravel the mechanisms at the base of aggressive fibromatosis.