RELAZIONE ATTIVITA' SCIENTIFICA

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PROGETTO DI RICERCA: Role of IGF-I/Fes/STAT3 axis in neuroblastoma metastasization.

Neuroblastoma (NBL) is the second most common extracranial solid tumor of childhood, accounting for 15% of cancer-related deaths. The most of cases occurs before age 2 years; as children get older, the number of new cases decreases. This tumor is slightly more common in males than females. NBL derives from embryonic neural crest cells of the peripheral sympathetic nervous system. About half of all NBL arises in the adrenal medulla, while the rest originate in paraspinal sympathetic ganglia in the chest or abdomen, or in pelvic ganglia (1, 2).
The biological characteristics and clinical heterogeneity of NBL make it unique among pediatric tumors. Indeed, it ranges from spontaneous regression in some patients (stage 4S) to rapid metastatic progression in others. Many genetic features identified in NBL, such as the ploidy status or oncogene amplification, correlate with clinical outcome. For instance, near-triploidy is associated with favourable prognosis, whereas *MYCN* oncogene amplification is linked with more aggressive tumors and negative outcome. Despite advances in multimodal therapy, prognosis for patients with high-risk NBL remains poor (1).
In the attempt to identify new targets for the therapy of this tumor, we have recently focused our attention on the expression and function of the non-receptor tyrosine kinase Fes in human NBL, since this protein is known to exert transforming or tumor suppressive functions depending on the district of expression (3).
Fes is a cytosolic protein encoded by the *c-fes* proto-oncogene, the cellular homolog of the transforming oncogene carried by several avian and feline retroviruses (4). The protein is expressed in myeloid hematopoietic and vascular endothelial cells, where it has been linked to signaling pathways controlling differentiation (5, 6). Nevertheless, Fes is also expressed in macrophages and neutrophils, with a role in inflammation (4); in embryonic tissues, where it is implicated in the earliest phases of development (7); in neurons, involved in pathways related to axon formation and sprouting (8). Furthermore, the kinase has been detected also in epithelial cells (in colon, kidney and in the mammary gland) (9, 10, 11).
As regards its biological functions, Fes has been historically viewed as a proto-oncogene because of its protein-tyrosine kinase activity (12, 13). Nevertheless, it has been recently described as a tumor suppressor in colorectal and in breast cancers (14). Given the variety of "cell type"-dependent roles played by Fes, we evaluated its function in the context of NBL tumor cell biology.
At first, by means of western-blot and confocal microscopy techniques, we evaluated the expression of Fes in a panel of human NBL cell lines different for morphology and positivity to *MYCN* amplification. These experiments revealed the existence of a rare population of cells expressing the kinase. Similarly to what observed in NBL cell lines, immunohistochemistry on NBL biopsies allowed us to detect the protein in a limited population of cells. Thus, we characterized this population, which resulted to display a more

aggressive phenotype. Indeed, by the MTT assay and experiments assessing the distribution of cells through the different phases of the cell cycle, we demonstrated an increased viability and proliferation of the Fes positive cells. Furthermore, these cells showed an increased capacity to form spheres in anchorage-independent conditions.

Recently, great interest has been directed to insulin-like growth factor (IGF) -I and its receptor, IGF-IR, given its important function in NBL progression (15). Thus, we decided to investigate the role of Fes along the IGF-I/IGF-IR system in NBL. Among the signaling pathways recruited down-stream of IGF-IR, it has been demonstrated that, in particular conditions, IGF-I may cause an activation of STAT3 *transcription factor (16)*. Our experiments revealed that, in NBL tumors, IGF-I may recruit STAT3 down-stream of its signaling pathway only in presence of functional Fes, thus influencing the metastatic potential of such tumor. Indeed, IGF-I causes a Fes-dependent activation of STAT3, thereby leading to the expression of factors having an impact on NBL microenvironment, i.e. IL10, VEGF.

IGF-I is also known for its considerable role in cell migration, an important process that contributes to cancer cell spread (17). By "wound-healing" assay, we observed that Fes activation enhances IGF-I induced motility of NBL cells.

In summary, our results provide the first evidence of the involvement of Fes in the progression of NBL, thus broadening the knowledge of NBL biology and of the interactions of this tumor with the host. Nevertheless, the identification of a specific molecular pathway could aid the development of novel therapies, more effective and less toxic than conventional ones.

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