FET proteins in cancer and development

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av

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Avhandlingen baseras på följande delarbeten:

I. Mattias K. Andersson, Anders Ståhlberg, Yvonne Arvidsson, Anita Olofsson, Henrik Semb, Göran Stenman, Ola Nilsson and Pierre Åman. The multifunctional FUS, EWS and TAF15 proto-oncoproteins show cell type-specific expression patterns and involvement in cell spreading and stress response. *BMC Cell Biology. 2008 Jul 11;9:37*.

II. Melker Göransson, Mattias K. Andersson, Claudia Forni, Anders Ståhlberg, Carola Andersson, Anita Olofsson, Roberto Mantovani and Pierre Åman. The myxoid liposarcoma FUS-DDIT3 fusion oncoprotein deregulates NF-κB target genes by interaction with NFKBIZ. *Oncogene. 2009 Jan 15;28(2):270-8. Epub 2008 Oct 13.*

III. Mattias K. Andersson, Melker Göransson, Anita Olofsson, Carola Andersson and Pierre Åman. Expression of FLT1 and its ligand PGF in *FUS-DDIT3* carrying myxoid liposarcomas suggests the existence of an intracrine signaling loop. *Manuscript*.

IV. Christoffer Bento*, Mattias K. Andersson*, Carola Andersson, Anita Olofsson och Pierre Åman. DDIT3 and the sarcoma fusion oncoprotein FUS-DDIT3 bind cyclin-dependent kinase 2. *Manuscript*.

* Dessa författare har bidragit likvärdigt till arbetet.

GÖTEBORGS UNIVERSITET

FET proteins in cancer and development

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Abstract

Chromosomal translocations leading to rearrangements of FET family genes (*FUS*, *EWSR1* and *TAF15*) are found in numerous human cancers. These genetic alterations result in the formation of fusion oncogenes that express potent chimeric oncoproteins able to promote tumor development. In order to further understand the function of the FET genes, we have characterized the expression of their encoded proteins products in human tissues and cells. By immunohistochemical analyses, we here demonstrate that the FUS, EWS and TAF15 proteins are expressed in a cell type-specific manner in human tissues. In experiments using cultured cells, we show that their expression is altered upon differentiation and that they localize to stress granules in response to cellular stress. Furthermore, FUS and TAF15 localize to spreading initiation centers upon early cell spreading. These results point to multiple cell type-specific functions for the FET proteins during both normal and stress conditions. We further attempted to elucidate the molecular mechanisms by which the aberrant FUS-DDIT3 protein gives rise to myxoid/round-cell liposarcoma (MLS/RCLS), a malignant soft-tissue tumor. FUS-DDIT3 expression results from a t(12;16)(q13;p11) translocation that is highly specific for MLS/RCLS and several studies have shown a causative role for FUS-DDIT3 in the development of these tumors. In FUS-DDIT3, the N-terminal parts of the RNA-binding FUS protein is fused to the entire DDIT3 protein, a transcription factor involved in endoplasmatic reticulum stress and programmed cell death. In the context of MLS/RCLS, the chimeric FUS-DDIT3 protein acts as an aberrant transcription factor able to deregulate multiple target genes. We have previously shown that FUS-DDIT3 and DDIT3 have opposing effects on *IL8* transcription in cells stably expressing these proteins. Here we demonstrate, by using multiple molecular methods, that FUS-DDIT3 interacts with the NF-κB system, specifically the NFKBIZ protein, and thereby activates *IL8* expression. These findings propose a role for inflammation-related processes in MLS/RCLS development. We further show that the growth factor receptor FLT1 and its ligand PGF are expressed in MLS/RCLS cells, which suggests the existence of an intracrine signaling loop in these cells. Moreover, through co-immunoprecipitation studies, we show that DDIT3 binds cyclin-dependent kinase 2 (CDK2), a protein involved in cell cycle regulation. This binding apparently alters the protein affinity of CDK2 and enhances its association with components of the cytoskeleton. The involvement of normal FET proteins in multiple regulatory pathways within a cell may explain why FET fusion genes are often the sole detectable abnormalities in their associated tumors. Specifically, the *FUS-DDIT3* gene studied in this thesis can promote several of the physical characteristics associated with cancer and thereby drive malignancy. In summary, the work included in this thesis suggests that agents which induce cellular differentiation, inhibit inflammatory processes (in particular the NF-κB system) or block FLT1 signaling may aid current treatments and thereby improve survival of patients afflicted with myxoid liposarcoma.

Keywords: FUS, EWS, TAF15, FUS-DDIT3, myxoid liposarcoma, NF-κB, NFKBIZ, FLT1, CDK2

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