Molecular analysis of the FET family fusion oncoprotein FUS-DDIT3

Akademisk avhandling

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av

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Avhandlingen baseras på följande delarbeten:

I. Alexandra Jauhiainen, Christer Thomsen, Linda Strömbom, Pernilla Grundevik, Carola Andersson, Anna Danielsson, Mattias K. Andersson, Olle Nerman, Linda Rörkvist, Anders Ståhlberg, Pierre Åman. Distinct Cytoplasmic and Nuclear Functions of the Stress Induced Protein DDIT3/CHOP/GADD153. *PLoS One, 2012. 7(4): e33208.*

II. Christer Thomsen, Sameer Udhane, Rikard Runnberg, Gerhard Wiche, Anders Ståhlberg, Pierre Åman. Fused in sarcoma (FUS) interacts with the cytolinker protein plectin: implications for FUS subcellular localization and function. *Experimental Cell Research, 2012. 318(5):653-61.*

III. Christer Thomsen, Pernilla Grundevik, Per Elias, Anders Ståhlberg and Pierre Åman. A binding motif in the N-terminal domains of the FUS, EWSR1 and TAF15 fusion oncoproteins mediates interactions with full-length FET family proteins. *Manuscript.*

IV. Christer Thomsen, Anders Ståhlberg and Pierre Åman. Fused in sarcoma (FUS) and the FUS-DDIT3 fusion oncoprotein interacts with the human SWI/SNF complex. *Manuscript.*

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Molecular analysis of the FET family fusion oncoprotein FUS-DDIT3

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Abstract

Chromosomal translocations leading to formation of tumor type specific fusion oncogenes are frequently found in human cancers. The FET family genes (*FUS*, *EWSR1* and *TAF15*) occur in translocations with genes encoding various transcription factors in sarcomas and leukemias. The resultant fusion genes encode chimeric protein products containing the N-terminal domain (NTD) of a FET family protein juxtaposed to the DNA binding domain of a transcription factor. The constitutively expressed fusion proteins act as abnormal transcription factors and are considered to be the primary initiating and driving factors of tumorigenesis. Myxoid liposarcoma (MLS) is defined by the t(12;16)(q13;p11) that cause the fusion of the *FUS* and *DDIT3* genes and expression of a chimeric FUS-DDIT3 oncoprotein. The aim of this thesis was to functionally characterize the FUS-DDIT3 fusion oncoprotein and the normal FUS, EWS, TAF15 and DDIT3 counterparts. To understand the involvement of DDIT3 in cancer and other pathologies we defined the genes and cellular functions it regulates. We observed that DDIT3 may localize to both the cell cytoplasm and nucleus. Microarray analysis revealed that cytoplasmic or nuclear DDIT3 regulate distinct sets of target genes. However, cytoplasmic or nuclear DDIT3 control the same cellular functions of which cell migration, proliferation, apoptosis/survival and cell cycle were most clearly affected. The majority of target genes were repressed supporting a role for DDIT3 as a dominant negative factor that may sequester and block the action of cytoplasmic and nuclear transcription factors. Subcellular localization is likely to be an important regulatory mechanism for control of DDIT3 activity. We further studied the molecular function of the FUS, EWS and TAF15 N-terminal domains with focus on their involvement in protein-protein interactions. The NTDs of FET proteins were found to bind the cytolinker plectin and FUS was shown to form complexes with plectin in the cell cytoplasm. Plectin expression was required for normal FUS function as plectin knockout cells revealed a shift in FUS nucleo-cytoplasmic distribution and a deregulation of FUS function in gene expression. Furthermore, we identified a conserved motif of the FUS, EWS and TAF15 NTDs that mediates interaction with full-length FET family proteins. The NTD of the FUS-DDIT3 fusion oncoprotein retained the capacity to bind wildtype FET proteins and disrupt their normal nuclear localization pattern. We also observed an involvement of wildtype FUS in the regulation of FUS-DDIT3 target genes. Finally, several components of the human SWI/SNF complex were isolated by pull-down with the FUS NTD. The SWI/SNF subunits Brg1 and ARID1A were further confirmed to form complexes with FUS-DDIT3 as well as wildtype FUS in the nuclei of MLS cells. SWI/SNF complexes control gene expression by modulating nucleosome positioning and are key players in cellular differentiation processes. Aberrant activity of SWI/SNF has further been linked with development of various cancers. Interaction with SWI/SNF is thus likely to be important for the impact of FUS-DDIT3 on gene regulation, differentiation processes and the transformed state of MLS.

Keywords: FUS-DDIT3, FUS, EWS, TAF15, DDIT3, myxoid liposarcoma, plectin, SWI/SNF

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