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First evidence for the FUS-CHOP fusion protein as a pro-metastatic molecule in human liposarcoma

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Liposarcomas are the most common soft tissue sarcomas, and although histology is the backbone for the diagnosis of all subtypes, differential diagnosis, clinical prognosis, and differential treatment remain a big clinical challenge. Recently, FUS-CHOP was identified as causal to the disease, but in view of the several transcripts and structural rearrangements, its identification remains a challenge. More important however, is that while this fusion protein has been found to be instrumental for specific oncogenic processes in liposarcoma, its ability to induce metastasis and the underlying mechanisms by which this can be achieved remain unknown.

On this background, this study was embarked upon to establish a concrete and robust method for the accurate identification of FUS-CHOP fusion transcripts, evaluate if FUS-CHOP is able to mediate increased migration, invasion and metastasis, and finally, to identify the target genes and downstream molecules implicated in FUS-CHOP mediated metastasis.

To tackle the issue of the detection of the different FUS-CHOP transcripts in a reliable and consistent manner, in both frozen and paraffin embedded tissues, we focused on the two most prevalent transcripts. We developed a novel approach using real-time PCR to identify and differentiate these fusion transcripts with the aim of improving accuracy over and above existing techniques. Our method was founded on the basis of transcript individualized primers and probes, which were designed to detect specifically the different variants in these tissues. This was achieved using a fluorescent labeled dye and quencher that considerably increased the detection sensitivity. Our results show that the method was more specific, sensitive, and superior to the widely used nested PCR. Primer amplification and probe detection of FUS-CHOP from genomic DNA of human, mouse, cocker spaniel and chicken sources all resulted in completely negative results, indicating this technique is specific for human RNA derived transcripts. In addition, the method was also valid in FFPE tissues, where RNA degradation is often a major problem. As an added benefit, this method also allows for the quantification of exact copy numbers of the detected transcripts.

The next major goal sought to dissect the functional role of FUS-CHOP particularly in the context of metastasis and preceding cascades of migration and invasion. To this end, we stably overexpressed this protein in SW872 liposarcoma and HT1080 fibrosarcoma cell lines, and were able to demonstrate that forced expression of FUS-CHOP significantly increases migration and invasion in these sarcoma cell lines, as well as enhance lung and liver metastasis in the in vivo chicken chorioallantoic membrane (CAM) model. We also demonstrate that FUS-CHOP enhances the expression of matrix-metalloproteinases -2, -7 and -9, and transactivates their promoters in vitro. Mutational analysis showed that C/EBP-β- (-769/-755), NF-κB (-525/-516) and CREB/AP-1 (-218/-207) sites were important for MMP-2; a C/EBP-β (-458/-444) site for MMP-7, and NF-κB (-604/-598), AP-1 (-539/-532) and AP-1 (-81/-72) for MMP-9 transactivation. Additionally a direct in vivo interaction of FUS-CHOP was observed in case of the MMP-2 promoter within region (-769/-207) using ChIP analysis. siRNA data revealed that MMP-2 expression is essential in the FUS-CHOP induced metastatic phenotype. MMP-2-mRNA and protein expression correlated significantly with FUS-CHOP positivity in 31 resected patient liposarcoma tissues.

Taken together, we have established a new method that offers an additional tool in the investigation of liposarcoma that may impact considerably on missed diagnosis and the accompanying clinical

relevance. Very significantly, however, we have for the first time provided substantial evidence for the FUS-CHOP chimeric oncoprotein as an inducer of metastasis that is due to the transcriptional induction of specific tumor-associated proteases. Lastly, insights gained from this study not only support a deeper understanding of the mechanistic properties of FUS-CHOP, but also open up new avenues for targeted therapy.