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FUS-CHOP Promotes Invasion in Myxoid Liposarcoma through a SRC/FAK/RHO/ROCK-Dependent Pathway[1]

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Abstract

Deregulated SRC/FAK signaling leads to enhanced migration and invasion in many types of tumors. In myxoid and round cell liposarcoma (MRCLS), an adipocytic tumor characterized by the expression of the fusion oncogene FUS-CHOP, SRC have been found as one of the most activated kinases. Here we used a cell-of-origin model of MRCLS and an MRCLS cell line to thoroughly characterize the mechanisms of cell invasion induced by FUS-CHOP using *in vitro* (3D spheroid invasion assays) and *in vivo* (chicken chorioallantoic membrane model) approaches. FUS-CHOP expression activated SRC-FAK signaling and increased the invasive ability of MRCLS cells. In addition, FAK expression was found to significantly correlate with tumor aggressiveness in sarcoma patient samples. The involvement of SRC/FAK activation in FUS-CHOP–mediated invasion was further confirmed using the SRC inhibitor dasatinib, the specific FAK inhibitor PF-573228, and FAK siRNA. Notably, dasatinib and PF573228 could also efficiently block the invasion of cancer stem cell subpopulations. Downstream of SRC/FAK signaling, we found that FUS-CHOP expression increases the levels of the RHO/ROCK downstream effector phospho-MLC2 (T18/S19)

Abbreviations: MRCLS, myxoid/round cell liposarcoma; FUS, fused in sarcoma; CHOP, C/EBP homologous protein; FAK, focal adhesion kinase; SFK, Src-family of protein kinases; RHO, RHOA/C GTPases; ROCK, Rho-associated coiled-coil-containing protein kinases 1 and 2; MLC2, myosin light chain 2; BMSC, bone marrow–derived mesechymal stem/stromal cell; FFPE, formalin-fixed, paraffin-embedded; CAM, chorioallantoic membrane; CSC, cancer stem cell.

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and that this activation was prevented by dasatinib or PF573228. Moreover, the ROCK inhibitor RKI-1447 was able to completely abolish invasion in FUS-CHOP–expressing cells. These data uncover the involvement of SRC/FAK/RHO/ROCK signaling axis in FUS-CHOP–mediated invasion, thus providing a rationale for testing inhibitors of this pathway as potential novel antimetastatic agents for MRCLS treatment.

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Introduction

Myxoid/round cell liposarcoma (MRCLS) is characterized by the recurrent translocations which fuse FUS (FUsed in Sarcoma; also termed TLS, Translocated in LipoSarcoma) or, less frequently, EWS to CHOP (C/EBP HOmologous Protein; also termed DDIT3, DNA Damage-Inducible Transcript 3). This type of liposarcoma shows a high tendency to recur locally or to metastasize to skeletal and pulmonary sites [1]. In addition, FUS-CHOP expression has been related with increased metastatic potential [2]. For MRCLS, treatment of metastatic disease mainly relies on cytotoxic drugs despite their limited clinical response [3]. Therefore, the study of the mechanisms involved in metastasis development may help to provide new therapeutic strategies to treat advanced and/or disseminated tumors more efficiently.

Nonreceptor protein tyrosine kinases, such as the focal adhesion kinase (FAK) and SRC proto-oncogene, are key signal transducers of a variety of cell surface receptors, including integrins and receptor tyrosine kinases [4–7]. Integrin stimulation induces FAK autophosphorylation at Y397, creating a high-affinity binding site for SRC. This association induces the autophosphorylation of SRC at Y419 and other conformational changes that lead full SRC activation [8]. Fully activated SRC can then further activate FAK by phosphorylation of its C-terminal domain (Y861 and Y925) and catalytic domain (Y576 and Y577) [5].

Deregulated activity of SRC-FAK signaling in cancer cells may lead to abnormal activation of several members of the Rho-family of GTPases, including RHOA/C (RHO) and RAC1, which are well-known regulators of cell migration and have been implicated in tumor cell invasion and metastasis [9,10]. In addition, the concurrent activation of signaling pathways like those mediated by PI3K-AKT, ERK, or JNK may induce prosurvival signals and the upregulation of proteolytic proteins that may contribute to the invasion process [4,5,9,11–15].

Tumor cells have been observed to migrate and invade as single cells, as loosely attached cell streams, or as compact and cohesive collectives by activating the above-mentioned signaling pathways. The type of cell migration is determined by both intrinsic and microenvironmental factors, such as the cell morphology, the degree of cell-cell adhesion, the existing supracellular signaling, and the physical properties of the microenvironment [12,16,17]. In the collective migration modes, one or several leader cells extend protrusions that generate traction forces and may induce proteolysis of the surrounding matrix. As a consequence, following cells are passively dragged behind along the migration track by cell-cell adhesion forming cell streams, small clusters, or well-organized masses [12,16,17]. During the invasion process, cells may switch from a collective to an individual mode of invasion. This transition occurs when the cell-cell and cell-extracellular matrix interactions are weakened and is influenced by several physical and molecular cues [12,18]. According to the type of cell movement, two main models of individual cell invasion with different requirements for Rho GTPs and proteolytic proteins have been described [9,12,14,19,20]. The "elongated-mesenchymal" mode is characterized by the formation of actin-rich protrusions at the front of the cell and is mainly driven by the activation of RAC1-induced signaling. The "rounded-amoeboid" movement is supported by high levels of actomyosin contractility which is induced by RHO-induced signaling. Thus, RHO activates the Rho-associated coiled-coil–containing protein kinases 1 and 2 (ROCK) resulting in the accumulation of the phosphorylated form of myosin light chain 2 (MLC2) at S19 which promotes actomyosin contractility [12]. Although better described for individual cell invasion models, mesenchymal and amoeboid morphologies and their associated signaling are also observed in collective cell invasion models [21].

In accordance with the protumorigenic role of altered SRC/FAK signaling or Rho GPase activity, these factors have been frequently found to be overexpressed in different tumor types and correlated with poor prognosis and increased invasive and metastatic potential [9,22–25]. Therefore, the clinical efficacy of several inhibitors with proved activity on SRC, like dasatinib, FAK, or ROCK, is being tested in many other solid tumors [7,26,27].

In sarcoma, several studies reported an increased activation of SRC in patient samples and cell lines derived from different sarcoma subtypes [28,29]. Furthermore, the inhibition of this activity with dasatinib reduced tumor cell growth and migration in liposarcoma, including myxoid liposarcoma, synovial sarcoma, and bone sarcoma cell lines [28–30]. Likewise, the expression and activation status of FAK have been related with decreased overall survival and metastasis-free survival and increased migration in osteosarcoma [31]. In addition, FAK inhibition resulted in decreased invasion, migration, and tumor growth in rhabdomyosarcoma [32]. Here, we used our previously developed MRCLS model [33,34], as well as an MRCLS cell line, to thoroughly characterize the mechanisms used by FUS-CHOP to promote cell invasion. Using both *in vitro* and *in vivo* approaches, we found that FUS-CHOP–induced invasive properties are mediated through the activation of SRC/FAK/RHO/ROCK signaling. These findings provide a rationale for testing inhibitors of this route as a novel therapeutic strategy for MRCLS.

Materials and Methods

Cell Types, Drugs, and Ethics Statement

Human BM-MSCs sequentially mutated with up to five oncogenic events were generated, characterized, and cultured as previously