Expression of the FUS-CHOP Fusion Protein in Primary Mesenchymal Progenitor Cells Gives Rise to a Model of Myxoid Liposarcoma

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**Abstract**

A subset of sarcomas is associated with specific chromosomal translocations that give rise to fusion genes believed to participate in transformation and oncogenesis. Identification of the primary cell environment that provides permissiveness for the oncogenic potential of these fusion genes is essential to understand sarcoma pathogenesis. We have recently shown that expression of the EWS-FLI-1 fusion protein in primary mesenchymal progenitor cells (MPCs) suffices to develop Ewing’s sarcoma-like tumors in mice. Because most sarcomas bearing unique chromosomal translocations are believed to originate from common progenitor cells, and because MPCs populate most organs, we expressed the sarcoma-associated fusion proteins FUS/TLS-CHOP, EWS-ATF1, and SYT-SSX1 in MPCs and tested the tumorigenic potential of these cells in vivo. Whereas expression of EWS-ATF1 and SYT-SSX1 failed to transform MPCs, FUS-CHOP–expressing cells formed tumors resembling human myxoid liposarcoma. Transcription profile analysis of these tumors revealed induction of transcripts known to be associated with myxoid liposarcoma and novel candidate genes, including PDGFA, whose expression was confirmed in human tumor samples. MPC FUS-CHOP and the previously described MPC EWS-FLI-1 tumors displayed distinct transcription profiles, consistent with the different target gene repertoires of their respective fusion proteins. Unexpectedly, a set of genes implicated in cell survival and adhesion displayed similar behavior in the two tumors, suggesting events that may be common to primary MPC transformation. Taken together, our observations suggest that expression of FUS-CHOP may be the initiating event in myxoid liposarcoma pathogenesis, and that MPCs may constitute one cell type from which these tumors originate. (Cancer Res 2006; 66(14): 7016-23)

**Introduction**

Two major categories of mesenchyme-derived cancer include hematopoietic malignancies and sarcomas. Sarcomas comprise bone and soft tissue tumors, several of which arise in children and young adults. Although they account for <10% of all malignancies, sarcomas are among the most aggressive forms of cancer in that they have a high metastatic proclivity and are typically refractory to conventional chemotherapy and radiation therapy. Currently, relatively little is known about their origin, biological properties, and pathogenesis.

Sarcomas can be subdivided into two subclasses according to the genetic events that underlie or accompany their development. One subclass is associated with multiple complex chromosomal deletions, translocations, and duplications (1–3), whereas the other typically carries specific “signature” mutations. The majority of these are chromosomal translocations that lead to the generation of fusion proteins, most of which behave as aberrant transcription factors (1–3). Although expression of these fusion proteins is believed to underlie the pathogenesis of the bone and soft tissue tumors with which they are associated, the mechanism whereby they transform cells are still poorly understood. Equally incomplete is the understanding of the type of cellular environment that allows the fusion proteins to display their potential oncogenic properties. A major challenge to elucidating the pathogenesis of sarcomas, therefore, is the identification of the fusion protein-primary cell combination that underlies their development (2, 3).

Recently, we found that the EWS-FLI-1 fusion protein, which is associated with 85% of Ewing’s sarcomas (also known as Ewing’s family tumors or EFT) can transform primary wild-type bone marrow–derived mesenchymal progenitor cells (MPCs) to form Ewing’s sarcoma–like tumors in mice (4). Moreover, EWS-FLI-1 expression in the absence of other pro-oncogenic events was sufficient to induce MPC transformation, suggesting that, in the appropriate cellular microenvironment, it may constitute the initiating event in EFT pathogenesis (4). Mesenchymal progenitor cells display a high degree of plasticity and can differentiate into osteocytes, adipocytes, neurons, and chondrocytes (5). Despite their bone marrow origin, MPCs can migrate to a broad range of tissues, including soft tissue compartments where most sarcomas develop (6). Based on these observations and the notion that sarcomas arise in pluripotent mesenchymal cells, we addressed the possibility that MPCs might provide the origin of other sarcomas associated with specific chromosomal translocations.

The EWS gene is the most frequent participant in chromosomal translocations that are specifically associated with sarcomas. Its fusion partners include FLI-1, ERG, ETVI, ETIV, and FEV (Ewing’s sarcoma); ATFI (clear cell sarcoma); WT1 (desmoplastic small round cell tumor); NR4A3 (myxoid chondrosarcoma); and CHOP/DDIT3 (myxoid liposarcoma; ref. 7). Interestingly, EWS shares functional properties with FUS/TLS (fused in sarcoma/translocated in sarcoma) that is also associated with sarcomas (8). Although less versatile than EWS, FUS can form fusion proteins with several partners, including ATFI (angiomatoid fibrous histiocytoma), ERG (Ewing’s sarcoma and acute myeloid leukemia), BBF2H7 (low-grade fibromyxoid...
sarcoma), and CHOP, (t(12;16)(q13p11) (myoid liposarcoma; ref. 9). EWS and FUS/TLS contain structural features that are consistent with their implication in RNA processing (10–13). The NH2-terminal domain of FUS/TLS binds to RNA polymerase II, whereas its COOH-terminal domain interacts with the transcription and translation factor Y-box binding protein 1. Within the FUS-CHOP fusion protein, the RNA-binding sequences of FUS are replaced by DNA-binding sequences and the basic leucine zipper domain of CHOP (11, 14). Similar to EWS, the NH2-terminal portion of FUS that forms the fusion proteins contains potent transactivation domains (10, 15). Thus, as in EWS fusion proteins, transcription is mediated by FUS sequences, whereas the DNA-binding motifs are provided by the fusion partner (10, 15).

In light of the transforming potential displayed by EWS-FLI-1 and the structural and functional similarities between EWS and FUS, we addressed the effect of the myoid liposarcoma–associated FUS-CHOP and clear cell sarcoma–associated EWS-ATF1 fusion proteins on MPC transformation and tumor development. Myxoid liposarcoma accounts for more than a third of liposarcomas, which amounts to about 10% of all adult soft tissue sarcomas (1). Recent studies suggest that myxoid liposarcoma express genes implicated in neural and osteocytic differentiation, supporting the notion that these tumors may be derived from mesenchymal progenitor cells (16, 17). The synovial sarcoma–associated fusion protein SYT-SSX1, which failed to display oncopgenic properties in MPCs in previous experiments (4), was used as a negative control for FUS-CHOP and EWS-ATF1.

Expression of FUS/TLS-CHOP in MPCs resulted in their transformation with development of myoid liposarcoma–like tumors. In contrast, MPCs expressing the EWS-ATF1 fusion protein failed to form tumors in vivo, as did SYT-SSX1 expressing cells. Our observations suggest that primary mesenchymal progenitor cells display selectivity permissiveness for sarcoma-associated fusion protein–mediated transformation, and that similar to EWS-FLI-1, FUS-CHOP expression can provide the single genetic event that is necessary and sufficient for the development of a defined soft tissue tumor from MPCs. Comparison of MPC-CHOP versus control cells identified distinct transcription profiles, consistent with different target gene repertoires of the fusion proteins and a common gene expression signature that may be linked to primary mesenchymal cell transformation.

**Materials and Methods**

**Cells.** MPCs were isolated from bone marrow of adult C57/6J wild-type mice according to the methods described (4) and cultured on fibronectin-coated plates (Sigma, St. Louis, MO) in medium containing 2% dialyzed FCS (Sigma), 10 ng/mL epidermal growth factor (Sigma), 10 ng/mL PDGF-BB (R&D Systems, Minneapolis, MN), and leukemia inhibitory factor that was produced by the CHO LIF720D, LIF-producing cell line. MPCs were tested for puromycin for a minimum of 5 days and a maximum of 10 days.

**plasmids** were sequenced to verify cDNA integrality.

**Retrovirus generation and infection.** Expression of hFUS-CHOPV5 and hEWS-ATF1 in MPCs was achieved using a retroviral gene delivery method. Briefly, ecotropic packaging 293 cells were transduced either with fusion genes containing pMSCV Puro or an empty pMSCV Puro vector, using Superfect transfection reagent (Qiagen, Valencia, CA). Supernatants were collected after 72 hours, diluted 1:1 with MRC medium containing 16 μg/mL of polybrene (Sigma), and added to six-well tissue culture plates containing 50% confluent MPCs. The plates were then centrifuged for 20 minutes at 1,800 rpm. RNA and proteins from infected cells were extracted at 24, 72 hours, and 10 days after infection. Expression of the fusion genes was tested at each time point by RT-PCR and Western blot (using the mouse anti-V5 or the rabbit anti-EWS antibody). The infected cells were selected as a bulk culture with 1.5 μg/mL puromycin for a minimum of 5 days and a maximum of 10 days.

**cDNA array hybridization.** Total RNA was extracted from each cell line using RNeasy Mini kit (Qiagen) according to the manufacturer’s recommendations. The quality and the integrity of total RNA were verified by an Agilent RNA 6000 nanoassay and by measuring the 260/280 absorbance ratio. Quality-tested total RNA was then amplified using the RiboAmp RNA Amplification kit (Arcturus, Mountain View, CA). After assessing the amplification by ethidium bromide gel electrophoresis, the amplified RNA was processed using a reverse transcription–based method of label incorporation to yield labeled cDNA. For each sample, 5 μg of amplified RNA were used in the cDNA probe synthesis with Cy5-dCTP or Cy3-dCTP (Amersham Biosciences, Amersham, United Kingdom) and random primers. Probes were purified using a Mini Elite PCR purification kit (Qiagen) and concentrated using Centricon YM-30 filters (Amicon, Millipore, Billerica, MA). Expression analysis was done using the NIA 17k clone set (18), Quantifoil support array. Hybridization was done in hybridization chambers (Corning Costar, Cambridge, MA) in a 64°C water bath for 16 hours.

**cDNA array analysis.** Following hybridization and washing, microarrays were imaged using a ScanArray 4000 scanner (Perkin-Elmer, Foster City, CA), and scanned slide images were converted to a tagged image file format. Fluorescence ratios for array elements were extracted by using ScanAlyze software, and further primary data analysis was done using crom.braju.sma routines in R statistical package. Cy5 (red) and Cy3 (green) signal intensities were used to calculate M and A for every spot on each array. M is a measure of differential gene expression and is calculated as the log 2 of the red and green intensity ratio (i.e., log 2 Cy5/Cy3). A is a measure of the signal strength that was calculated as the mean of the log 2 red and green intensity: (log 2 Cy5 + log 2 Cy3) / 2. M values were normalized using the library sma in the statistical software package R by the within-print-tif group Lowess normalization procedure (19). Quality control of slide hybridization was done using variables described on the corresponding web site.4

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4 For accurate description, see http://intranet.liren.isb-sib.ch/microarrays/clones.html.
5 http://rana.lbl.gov/EisenSoftware.htm.
6 http://www.maths.lth.se/help/R/.
7 http://www.r-project.org/.
8 http://www.nml.ch/daf/.

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For accurate description, see http://intranet.liren.isb-sib.ch/microarrays/clones.html.
Statistical analysis of the expression data. For each time point and cell line, five m17k microarrays (among which two were dye swaps) were done comparing hFUS-CHOPV5 expressing with empty vector control cells. Expression data for each time point and cell line were analyzed with standard one-sample, two-sided t tests applied to the logarithm of the ratio of the expression levels of the hFUS-CHOPV5 and the control sample. The null hypothesis is that the mean of such a logarithm is 0, and the alternate hypothesis is that the mean is not equal to 0.

The use of a standard statistical test (as opposed to methods based on cut-off on fold-change) allowed us to estimate the false discovery rate (FDR) of the lists of induced and repressed genes. This was done using the Benjamini-Hochberg method (20), in which the clones are sorted by increasing t test P, and the list thus obtained is truncated in correspondence of the last gene for which the FDR estimator is lower than a preset value. The FDR estimator is given by \( P \leq \frac{N \times C}{n} \), where \( N \) is the total number of clones analyzed, \( C \) is the t test P of the gene, and \( n \) is its position in the list sorted by increasing P.

Western blot analysis. Cells were lysed for 20 minutes on ice in a nuclear lysis buffer containing 50 mmol/L Tris (pH 7.5), 0.5 mmol/L NaCl, 1% NP40, 1% sodium deoxycholate, 0.1% SDS, 2 mmol/L EDTA, and complete protease inhibitors (Roche, Basel, Switzerland), and the lysates were then sonicated on ice thrice for 30 seconds. Samples were subjected to SDS-PAGE, and proteins were blotted onto polyvinylidene difluoride membranes (Millipore). Immunostaining was done after blocking with 5% nonfat dry milk, and bands were detected using a chemiluminescent substrate kit (Amersham Biosciences) according to the manufacturer’s recommendations. Primary monoclonal mouse anti-V5 epitope (Invitrogen) or polyclonal rabbit anti-EWS (Bethyl Laboratories, Montgomery, TX) antibodies and secondary horseradish peroxidase–conjugated goat anti-mouse or anti-rabbit (Bio-Rad, Hercules, CA) antibodies were used.

Real-time quantitative RT-PCR. cDNA was obtained using an M-MLV reverse transcriptase and RNase H minus (Promega, Madison, WI). Typically, 500 ng of template total RNA and 250 ng of random hexamers were used per reaction. Real-time PCR amplification was done using a Taqman PCR Universal mastermix and Assays-On-Demand gene expression products in an ABI Prism 7700 instrument (Applied Biosystems, Foster City, CA). Relative quantitation of target, normalized with an endogenous control (cyclophilin A), was done using a comparative C\( \text{t} \) or a standard curve method (Applied Biosystems).

In vitro NVP-AEW541 sensitivity assays. For the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, MPCs were cultured in 24-well plates and treated with normal medium, 0.5 mmol/L NVP-AEW541, or solvent (DMSO) for 10 days. Cell sensitivity for the drug was tested with CellTititer Aqueous Non-Radioactive Cell Proliferation Assay (Promega) according to the manufacturer’s recommendations.

Tumorigenicity assays. Five-week-old BALB/c/\( \text{H} \)NadHsc-SCID mice were obtained from Harlan (Indianapolis, IN). For each MPC cell population, 12 mice were injected s.c. with \( 1 \times 10^6 \) cells stably expressing either hFUS-CHOPV5, hEWS-ATF1, hSYT-SSX1, or empty vector. Tumor growth was monitored weekly, and tumor-bearing mice were euthanized 6 weeks after injection. Six mice were injected s.c. with \( 1.5 \times 10^6 \) cells from each independent MPCs fused to CHOP tumor-derived cell population and sacrificed 2 weeks after injection. All tumors were resected at autopsy and sectioned for histology. All experimental protocols involving mice were approved by the Etat de Vaud, Service Vétérinaire, authorization no. VD1477.0.

Tumor explants. Tumor-bearing mice were euthanized, and the tumors were excised, disaggregated in 4 mL of PBS supplemented with 10% fetal bovine serum + Ca\(^{2+} \) and Mg\(^{2+} \), and resuspended for 40 minutes at 37° C in 15 mL of an enzymatic cocktail containing 2 mg collagenase VIII + 30 mg trypsin in 30 mL of PBS. The supernatants were then filtered and centrifuged for 6 minutes at 400 × g, and the resulting cellular pellets were plated in MPC medium without LIF; containing 1.5 μg/mL puromycin for tumor cell selection.

Immunohistochemistry. Paraffin-embedded sections of MPCs fused to CHOP, and MPCs fused to CHOP expressing FUS-ATF1 or SYT-SSX1 were stained with mouse anti-human CD99 (1:40 dilution; Signet Laboratories, Dedham MA) and neural-specific enolase (NSE; 1:100 dilution; DAKO, Glostrup, Denmark) monoclonal antibody, or goat anti-human PDGF-A (1:100 dilution; R&D Systems). The latter antibody was also used to stain the human sarcoma paraffin sections. Horseradish peroxidase staining was done using biotin-conjugated horse anti-mouse or rabbit anti-goat immunoglobulin (DAKO) and revealed with a DAKO 3,3′-Diaminobenzidine kit (DAKO).

Results

MPCs expressing FUS-CHOP form tumors resembling myxoid liposarcoma. The FUS-CHOP, EWS-ATF1, and SYT-SSX cDNAs were isolated by RT-PCR from total RNA derived from frozen surgical specimens of human myxoid liposarcoma, clear cell sarcoma, and synovial sarcoma, respectively. The FUS-CHOP cDNA was amplified from a tumor containing the fusion of exon 5 of FUS to exon 2 of CHOP (type 2 fusion; ref. 1), which is seen in about 70% of cases, and sequences encoding the v5 tag were added to the 3′ end. Each cDNA was then inserted into the pMSCV retroviral vector, and the corresponding constructs were used to produce retroviruses and infect MPCs as described previously (4). Mesenchymal progenitor cells were derived from the bone marrow of wild-type C57BL/6 mice as described in Materials and Methods and characterized by a CD45-negative, CD11b-negative, CD44-positive, Thy1-positive, CD117 weakly positive, CD13 weakly positive, and Sca1 strongly positive phenotype (21, 22). Upon stimulation with appropriate growth factors, these cells differentiated into adipocytes, myocytes, chondrocytes, and neurons (21, 22) but failed to produce tumors in immunocompromised mice.

Bulk cultures of retrovirally infected MPC were selected for further experiments. Expression of each fusion protein was verified by Western blot analysis of lysates of the corresponding MPCs using anti-v5 or anti-EWS antibodies. All three fusion proteins were observed to be stably expressed (Fig. 1), and no major morphologic changes were noted in any of the retrovirally infected cells.

Ten days after infection each of the bulk MPC cultures was injected s.c. into a minimum of 12 severe combined immunodeficient mice. Three weeks following injection, all 12 mice that had received MPCFUS-CHOP cells developed visible tumors (Fig. 2A), and four animals were sacrificed for tumor analysis each week thereafter. By contrast, MPCEWS-ATF1 and MPCSYT-SSX failed to form tumors as late as 4 months after injection, when the experiment was terminated. The experiment was repeated with fresh batches of MPC, with and without the v5 tag and using different selection periods with identical results.

Human myxoid liposarcoma typically display variable cellularity, often containing lipogenic tumor cells surrounded by a myxoid stroma and numerous dilated capillaries (23). Histologic analysis revealed that the MPCFUS-CHOP tumors were composed of small, occasionally vacuolated cells embedded in a richly vascularized myxoid matrix (Fig. 2B; Supplementary Data S3), reminiscent of human myxoid liposarcoma (Fig. 2B; Supplementary Data S3). To determine whether the observed phenotype undergoes modifications with tumor progression, tumor-derived MPCFUS-CHOP cells were disaggregated and reinjected into mice. Although the second round tumors appeared more rapidly than their predecessors (Fig. 2A), their growth rate was only slightly higher than that of late stage first round tumors, whereas their histologic phenotype was comparable (data not shown). Importantly, expression of the FUS-CHOP protein was maintained in the first- and second-round tumor-derived cells (Fig. 1; data not shown). One possible explanation for the more rapid outgrowth of these second round tumors may be that they were derived from cells that had adapted to the s.c. microenvironment, tumor take was facilitated. Although it is possible that these tumors incurred additional
genetic modifications that favor survival and growth, gene expression profile comparison between first- and second-round tumors failed to reveal changes that might reflect any obvious genetic events (data not shown).

Transcriptome modifications induced by FUS-CHOP in MPCs and MPCFUS-CHOP tumor cells. To determine the effect of FUS-CHOP on gene expression in MPCs, we compared transcription profiles of cells infected with FUS-CHOP containing retrovirus to those of corresponding cells infected with empty virus 24 and 72 hours as well as 14 days after infection. RNA was obtained from two independently FUS-CHOP–infected and two independently empty virus-infected cell populations, and expression of 17,000 cDNA clones was compared using the NIA-17K mouse cDNA array (18). Four microarrays were used to assess expression profile changes in each cell population. Expression data for each clone present on the microarray were analyzed with standard one-sample, two-sided t tests applied to the logarithm of the ratio of the expression levels of the MPCFUS-CHOPV5 and MPCVector sample. This approach helped identify sets of FUS-CHOP–induced and repressed genes (Supplementary Data S1), and the false discovery rate (FDR) of each gene set was estimated by the Benjamini-Hochberg method as described in Materials and Methods. At 24 hours, 652 and 483 genes were respectively induced and repressed in response to FUS-CHOP expression with an FDR of 5%, whereas 610 and 555 genes, or EWS-ATF1 (E-A) expressing vectors.

To obtain clues as to the mechanisms that underlie FUS-CHOP–induced tumorigenicity, we compared the gene expression profile of cells derived from the first-round MPCFUS-CHOP tumors to their preinjection counterparts. RNA was obtained from two independently FUS-CHOP–infected and two independent tumor-derived cell populations, and expression of 17,000 cDNA clones was compared using the NIA-17K mouse cDNA array as above. Five microarrays were used to assess expression profile changes in each cell population. The gene expression pattern of MPCFUS-CHOP tumors compared with that of MPCFUS-CHOP cells before injection included repression of CTGF, PERP, and TFF1 and induction of transcripts encoding growth factors (PDGFA and HGF), cytokines (IL6), growth factor receptors (MET), cell cycle regulators (CDK4 and MDM2), proteolytic enzymes (MMP-11, CTSD, and PLAT), and factors implicated in adipocyte differentiation, including ADFP, FASN, HMGCR, and RGS2 (Fig. 3A–C; refs. 24, 25). Possibly explaining, in part, the histologic phenotype of the tumors. Among these genes, CD24, LXX, CD1D1, PLTP, DAF1, HOXD3, and ADM were found to be induced in NIH-3T3 cells by FUS-CHOP (Fig. 3B; ref. 17). Association of the cell cycle regulators CDK4, MDM2 (26), the oncogene MET (27), fatty acid synthase (FASN; ref. 24), and IL6 (28) with myxoid liposarcoma (Fig. 3C) was established by immunohistochemical studies on myxoid liposarcoma samples.

Induction of PDGFA had not been reported in myxoid liposarcomas previously, which prompted us to validate its expression by quantitative real-time PCR analysis and assess PDGFA expression in MPCFUS-CHOP tumors and human myxoid liposarcomas by immunohistochemistry. Consistent with the quantitative real-time PCR data (Fig. 3C), MPCFUS-CHOP tumors stained positively with anti-PDGFA antibody as did all 10 samples of human myxoid liposarcoma tested (Fig. 3D). By contrast, synovial sarcoma, Ewing’s sarcoma, and chondrosarcoma did not display PDGFA expression as detected by immunohistochemistry, whereas alveolar rhabdomyosarcoma showed only marginal reactivity with the antibody (Fig. 4).

MPCs provide a unique model system to study myxoid liposarcoma and EFT development. Having recently observed that MPCs expressing the EWS-FLI-1 fusion protein form Ewing sarcoma–like tumors in mice (4), we compared the effect of FUS-CHOP and EWS-FLI-1 on MPCs. We first assessed the expression profile changes of cells infected with each fusion gene at 24 hours, when the number of transcripts displaying expression change was the highest (4). As might be expected, major differences in the

Figure 1. Western blot analysis of FUS-CHOP, SYT-SSX1, and EWS-ATF1 fusion protein expression. A, left, FUS-CHOPV5 expression in MPCs 10 days after infection with either empty (lane E) or FUS-CHOPV5 expressing vector (lane F-CV5). Right, expression of the FUS-CHOPV5 fusion protein in two independent MPCFUS-CHOPV5–derived cell populations (T1-1 and T1-2). B and C, expression of the Syt-SSX1V5 (B) and EWS-ATF1 (C) chimeric proteins in MPCs 10 days after infection with empty (E), SYT-SSX1V5 (S-SV5), or EWS-ATF1 (E-A) expressing vectors.

Figure 2. Growth and histology of MPCFUS-CHOP tumors. A, tumor growth in SCID mice. B, histology of the MPCFUS-CHOP tumors is reminiscent of that of human MLPS, characterized by the presence of round cells surrounded by an abundant, and richly vascularized myxoid stroma. H&E staining. Magnification, ×100.
composition of the induced and repressed transcripts in response to the two fusion proteins were observed (Supplementary Data S1). To further highlight this difference, we compared the number of genes that displayed similar or opposite expression changes in response to the two fusion proteins. We found that at 24 hours, only 17 transcripts displayed similar movement in response to both fusion proteins: 10 being induced and 7 repressed (Supplementary Data S1). By contrast, 124 genes that were induced in EWS-FLI-1–infected cells were repressed in FUS-CHOP–infected counterparts, whereas 212 transcripts displayed the inverse behavior (Supplementary Data S1).

Consistent with the observed difference in the early response of MPCs to FUS-CHOP and EWS-FLI-1, the morphology and transcription profile of MPCEWS-FLI-1 and MPCFUS-CHOP tumors were divergent. Histologically, the tumors were distinct, with the former being characterized by sheets of small round blue cells and the latter by small often vacuolated cells with a richly vascularized myxoid matrix (Fig. 5). NSE expression, which is associated with EFTs and MPCEWS-FLI-1 tumors, was not observed in MPCFUS-CHOP tumors (Fig. 5). Conversely, MPCFUS-CHOP tumor-associated PDGFα expression was lacking in MPCEWS-FLI-1 counterparts (Fig. 5).

Comparison of MPCEWS-FLI-1 and MPCFUS-CHOP tumor transcriptomes showed marked differences with respect to genes that bear potential relevance to the pathogenesis of each tumor type (Supplementary Data S2). Importantly, several genes, including the adipocytic differentiation markers FASN, HMGCR, and BGS2, that were induced in MPCEWS-FLI-1 tumors but not in MPCFUS-CHOP cells and tumors, and PDGFα being induced in MPCFUS-CHOP tumors but repressed in MPCEWS-FLI-1 counterparts (Fig. 6B; Supplementary Data S2). Consistent with these observations, MPCFUS-CHOP tumors did not recapitulate the high sensitivity displayed by MPCEWS-FLI-1 tumors to IGF-1R blockade by the pyrrolo[2,3-d]pyrimidine derivative small molecular weight kinase inhibitor AEW541 (refs. 4, 29; Fig. 6C).

Interestingly, several genes found to be induced or repressed in tumors derived from transformed MPCs were shared by MPCEWS-FLI-1 and MPCFUS-CHOP tumor cells. They included YAP, BIRC2, TGFBI, which were induced, and LUM, ALCAM, JAM2, JAM3, CAH11, FATH, and CALDESMON1, which were repressed in both tumor types (Fig. 6D). Induction and repression of selected transcripts within this group, including ALCAM, JAM2, LUM, BIRC2, and YAP, were validated in MPCFUS-CHOP tumor cells by quantitative RT-PCR analysis (Fig. 6E).
FUS-CHOP has been observed to induce transformation in cell type–specific fashion (9), suggesting that permissiveness for its oncogenicity is a function of the cellular context. Our present work provides evidence that primary MPCs provide a permissive environment for FUS-CHOP–mediated transformation, and that MPCs expressing FUS-CHOP form tumors resembling human myxoid liposarcoma. Coupled to our recent finding that EWS-FLI-1 expression in MPCs leads to the formation of EFT-like tumors, these observations raise the possibility that myxoid liposarcoma and EFTs may originate from the same or closely related cells, albeit at different locations within the body. The preferential bone and soft tissue localization of EFTs and myxoid liposarcoma, respectively, may depend, in part, on local microenvironment-dependent modulation of MPC susceptibility to transformation by the corresponding fusion proteins and in part on the ability of the transformed cells to adapt to constraints imposed by the physiologic properties of the surrounding tissue.

Similar to the effect of EWS-FLI-1, expression of FUS-CHOP alone was sufficient to transform MPCs and induce them to form tumors in vivo. Although it is widely held that development of solid tumors requires an average of three genetic events (30), many of the experiments on which this view is based were done in differentiated cells with limited plasticity and potential for self-renewal. Mesenchymal progenitor and hematopoietic stem cells retain high self-renewal proclivity, survival capacity, and migratory and invasive properties (31, 32), all of which constitute features of malignant cells. Our present observations are consistent with the possibility that in such an environment a single, appropriately targeted, genetic event may suffice to induce transformation. Attempts to develop transgenic models of liposarcoma using FUS-CHOP constructs driven by adipocyte-specific promoters failed, further suggesting that FUS-CHOP may require a mesenchymal progenitor/stem cell environment to exert its oncogenic potential. Consistent with this notion, transgenic mice engineered to express FUS-CHOP under the control of the ubiquitous E1Fa promoter, which we found to be functional in MPCs (data not shown), developed liposarcomas that resemble their human counterpart (33). Up-regulation of neuronal transcripts PN-1, Neuronatin, and RET in myxoid liposarcoma cells compared with normal adipocytes (16, 34) add further support to a mesenchymal progenitor/stem cell origin of myxoid liposarcoma.

Transcriptome analysis of MPCFUS-CHOP tumor cells revealed up-regulation of genes observed to be associated with myxoid liposarcoma and FUS-CHOP–transfected NIH-3T3 cells. Several of these genes, including MDM2 (26), CDK4 (35), and HGFR (c-met; ref. 27), are believed to participate in myxoid liposarcoma development. The same may hold true for PDGFa, which has a potent promigratory and growth-promoting effect on mesenchymal cells, and whose association with myxoid liposarcoma was a novel discovery in this study. Although PDGFa expression is associated with a variety of malignancies (36), its robust induction in MPCs by FUS-CHOP expression and presence in myxoid liposarcoma suggests a possible role in the pathogenesis of these tumors, much as IGF-1 seems to be implicated in the pathogenesis of Ewing’s sarcoma growth, without being EFT specific.

Our present observations cannot predict which of the induced/suppressed genes are direct FUS-CHOP targets and which are indirectly affected by FUS-CHOP, possibly secondary to FUS-CHOP–induced differentiation or transformation. However, it is likely that a combination of both direct and indirect targets contributes to MPCFUS-CHOP tumor development. Moreover, induction of genes that are associated with myxoid liposarcoma and proposed to participate in its pathogenesis supports the notion that MPCFUS-CHOP tumors constitute a relevant model of myxoid liposarcoma.

Direct comparison of the effect of FUS-CHOP and EWS-FLI-1 expression in MPCs revealed major differences not only in the altered transcript repertoire but in the inverse response of >300 genes to the two fusion proteins. Comparisons of MPCFUS-CHOP to MPCEWS-FLI-1 tumors further highlighted the difference in...
EWS-FLI-1- and FUS-CHOP-mediated events. In addition to different histologic phenotypes, the tumors displayed distinct gene expression profiles consistent with the notion that despite a shared cell of origin, different signaling pathways underlie the pathogenesis of each tumor type. Thus, MPCFUS-CHOP tumors expressed PDGFα, HGF, and GFRA1 but did not up-regulate IGF-1 and, in contrast to MPC EWS-FLI-1 tumors, displayed only moderate sensitivity to IGF-1R blockade (4). Moreover, adipose tissue differentiation genes associated with MPCFUS-CHOP tumors, including FASN, HMGCR, and RGS2, were repressed in MPC EWS-FLI-1 tumor cells consistent with the notion that EWS-FLI-1 blocks adipocyte differentiation in MPCs (37). The absence of peroxisome proliferator-activated receptor γ (PPARγ) induction suggests a partial adipose lineage differentiation block in MPCFUS-CHOP tumors as well, consistent with the proposed effect of FUS-CHOP on PPARγ activity (32, 38–40).

Interestingly, many of the reported EWS-FLI-1 target genes and myxoid liposarcoma–associated genes were expressed in first round tumor cells but not in MPCs bearing the fusion proteins before injection. A possible explanation may be that a combination of transformation and in vivo growth conditions enables EWS-FLI-1 and FUS-CHOP bearing MPCs to express direct and indirect target genes of their respective fusion proteins. This notion is supported by the observation that EWS-FLI-1 expression resulted in the induction of several target genes in NIH-3T3 cells (41) but not in primary fibroblasts (42, 43).

Whereas distinct transcription profiles of MPC EWS-FLI-1 and MPCFUS-CHOP tumors could be anticipated, based on different
target gene specificity of the respective fusion proteins, the shared expression profile of several genes that may be relevant to oncogenesis were unexpected. Both tumor types were associated with induction of yes-associated protein (YAP) and BIRC2, which are believed to be involved in promoting cell survival. Both tumors also displayed repression of genes encoding lumican, fat-associated tumor suppressor, caldesmon, and the adhesion receptors ALCAM, Jam-2 and Jam-3, and cadherin 11. Repression of lumican, a small secreted protoglycan, has been observed to augment tumor cell proliferation (44, 45), whereas loss of adhesion receptors may facilitate cell migration and invasion. The observed induction of genes common to the two tumors may constitute a transcription profile that reflects important events in mesenchymal cell transformation. It is noteworthy that this putative expression signature was unaffected by the duration of tumor growth, being detected in MPC-FUS-CHOPT1 and MPC-EWS-FLI-T1 respectively, 6 and 15 weeks following injection of the corresponding cells.

Taken together, our observations support the notion that expression of FUS-CHOP in the appropriate cellular environment may be the initiating event in myxoid liposarcoma development. Coupled to our recent report that EWS-FLI-1 expression transforms primary MPCs, the present study indicates that transformation of MPCs may be induced by single, selected genetic events, and that MPCs may provide a common origin of both EFTs and myxoid liposarcomas. MPC-derived tumor models may facilitate elucidation of the mechanisms, whereby FUS-CHOP and EWS-FLI-1 transform primary mesenchymal cells and provide new insight into the molecular pathogenesis of some of the most aggressive malignancies.

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