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Induction of a secreted protein by the myxoid liposarcoma oncogene

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ABSTRACT **The TLS-CHOP oncoprotein, found in the majority of human myxoid liposarcomas, consists of a fusion between the transcription factor CHOP/GADD153 and the N terminus of an RNA-binding protein TLS/FUS. Clinical correlation and *in vitro* transformation assays indicate that the N terminus of TLS plays an important role in oncogenesis by TLS-CHOP. Until now, however, the only activity attributed to the oncoprotein is that of inhibiting the binding of transcription factors of the C/EBP class to certain adipogenic target genes, a function that TLS-CHOP shares with the nononcogenic CHOP protein. Here we report the isolation of a gene, DOL54, that is activated in primary fibroblasts by the expression of TLS-CHOP. DOL54 is expressed in the neoplastic component of human myxoid liposarcomas and increases the tumorigenicity of cells injected in nude mice. Activation of *DOL54* requires an intact DNA-binding and dimerization domain in TLS-CHOP, a suitable cellular dimerization partner, and depends on the TLS N terminus. Normal adipocytic differentiation is associated with an early and transient expression of *DOL54,* and the gene encodes a secreted protein that is tightly associated with the cell surface or extracellular matrix. TLS-CHOP thus leads to the unscheduled expression of a gene that is normally associated with adipocytic differentiation.**

Most, if not all, human myxoid and round cell liposarcomas are associated with chromosomal translocations (1). These chromosomal translocations lead to gene fusions that encode chimeric oncoproteins consisting of an N terminus contributed by one of two related genes, *TLS* or *EWS,* and a C terminus contributed by the *CHOP* gene (2–5). Both components of the resulting fusion oncoprotein are important to its transforming activity (6), but the precise role of each remains to be fully elucidated.

CHOP, the invariant component of these oncoproteins, is a small nuclear protein that avidly forms heterodimers with members of the C/EBP family of transcription factors (7). These heterodimers exhibit two distinct activities: On the one hand they interfere with the ability of the C/EBP partner to bind to certain "classical" binding sites and thus inhibit the activity of a set of C/EBP target genes (7–10). At the same time, the C/EBP-CHOP heteromeric complex is directed to a special subset of C/EBP binding sites from which they activate a set of target genes (10–13). Members of the C/EBP family are known to have an essential role in adipose tissue development (14–19), and TLS-CHOP blocks differentiation of adipogenic precursors in tissue culture (10, 20). Whereas interference with the normal process of differentiation may contribute to the oncogenic potential of TLS/EWS-CHOP fusion proteins, it is a property that they share with the nontransforming germline *CHOP* gene product (6, 8). Furthermore, genetic analysis of TLS-CHOP assigns an essential role to the CHOP DNA-binding domain in oncogenesis (6). Because this domain is not required for the inhibitory effect of CHOP on C/EBP binding to classic target genes (8, 11), activation of nonclassic target genes is likely to play a role in the transformation process.

CHOP expression is tightly regulated. Under normal conditions the gene is repressed, and CHOP protein and mRNA are absent from cells. However, stress markedly activates *CHOP* transcription (21–24). Stress also regulates CHOP activity through induced phosphorylation of the protein and consequent enhancement in its transactivation potential (25). In contrast with *CHOP*, *TLS* and *EWS* are constitutively activated genes (26), and their functionally interchangeable 5′ contribution to the fusion oncogenes encodes a peptide with strong transcriptional activation potential (6, 27–29). These observations support a model whereby the *TLS/EWS-CHOP* fusion genes represent gain-of-function mutations of *CHOP* that deregulate both gene expression and protein activity. Thus, TLS/EWS-CHOP would be predicted to activate certain downstream target genes. Here we describe the cloning of a gene that is specifically activated by oncogenic CHOP fusion proteins but not by nononcogenic derivatives. Its normal pattern of expression suggests a relationship to the process of adipocytic differentiation.

MATERIALS AND METHODS

Construction of an Allele Conditionally Expressing TLS-CHOP in the Mouse Germline. The 5′ and 3′ homology arms for targeting the *Tls* locus correspond to the *Bal*I–*Bsp*HI and *Mun1–Xba*I murine genomic fragments. The replacement cassette consisted of a *loxP* site (30), the *Neo* gene of Tn5, and a second *loxP* site followed by the entire human *CHOP* coding region derived from the *TLS-CHOP* cDNA (31). Terminator sequences were from the herpes simplex thymidine kinase gene. The junctions of the fragments were organized such that a TLS-Neo fusion protein would be encoded by the targeted allele before site-specific recombination at the *loxP* sites (*Tls.m2* allele), whereas a TLS-CHOP fusion protein would be encoded after such recombination. W4 embryonic stem cells were transfected and G418 resistant clones isolated and genotyped by PCR as indicated in Fig. 1*A*. Eight out of 20

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Abbreviations: MEF, mouse embryonic fibroblasts; RDA, representational difference analysis; MSF, megakaryocyte stimulatory factor; CHO, Chinese hamster ovary cells.
Data deposition: The sequence of human DOL54/MSF reported in this paper has been deposited in the GenBank database (accession no. HSU70316).
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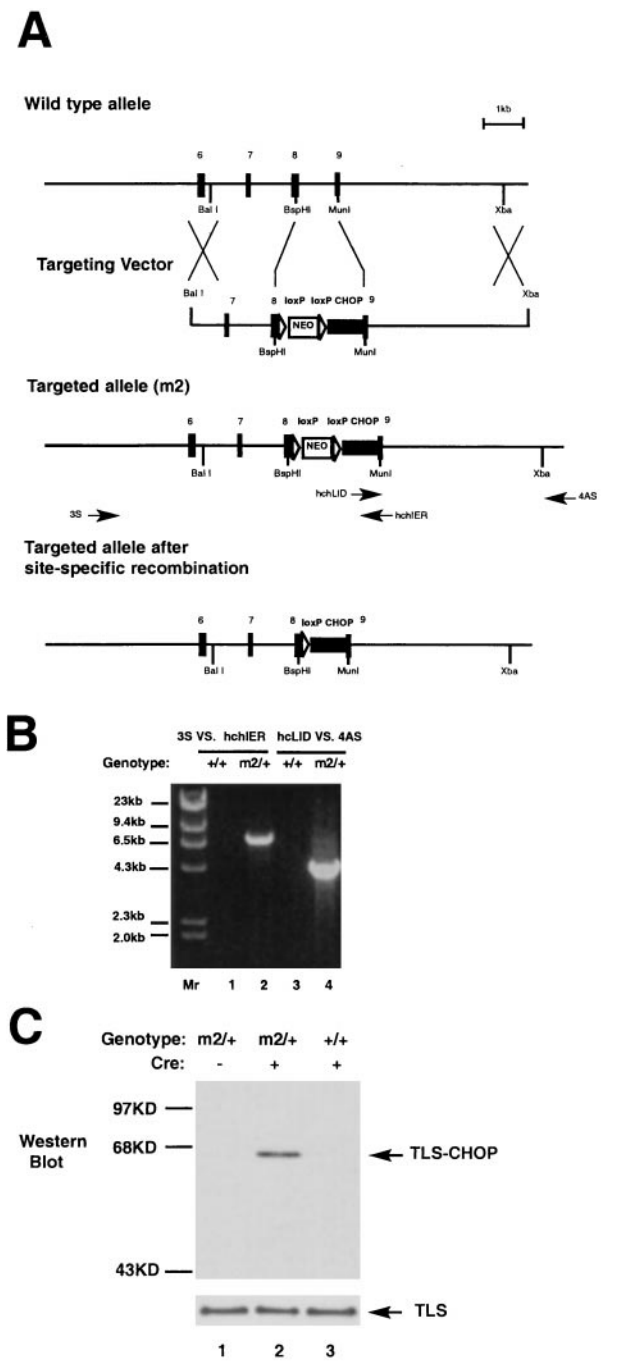

FIG. 1. A conditional allele expressing *TLS-CHOP*. (*A*) Targeting vector and targeted allele of *Tls* ("m2") before and after Cre-mediated site-specific recombination. The *loxP*-flanked *Neo* cassette and juxtaposed human *CHOP* coding region represent an in-frame interruption of the *Tls* gene. Before Cre-mediated recombination, the allele encodes a TLS-Neo fusion protein, whereas after recombination it encodes TLS-CHOP. (*B*) Genomic PCR analysis of the targeted allele. The 3S and 4AS primers lie outside the targeting vector (horizontal arrows). (*C*) Site-specific recombination-dependent expression of TLS-CHOP. Shown is a Western blot of whole-cell extracts from embryonic fibroblasts with (m2/+) or without (+/+) the mutant *Tls* allele that had been transduced with a Cre-expressing retrovirus. Position of the TLS-CHOP protein is indicated by the arrow. TLS serves as a loading marker.

G418-resistant clones tested were properly targeted and three of those produced germline chimeras. These were bred to FVB/n females to produce isogenic F1 progeny from which day 14 mouse embryonic fibroblasts (MEFs) were derived by standard techniques (32).

***In Vivo* Neo Excision and Characterization of the TLS-CHOP$^+$ Cells.** The Cre-encoding retrovirus was produced by ligating the Mlu1 fragment of pMC.Cre into the Bgl2-R1-digested MSCVhph vector (33), from which the selection marker had been deleted. Recombinant retrovirus was produced by transient transfection of COS1 cells according to an established method (34). MEFs were transduced with the Cre-encoding retrovirus and 48 hr later whole-cell lysates for TLS-CHOP immunoblot were prepared, or the cells were fixed and immunostained for TLS-CHOP by using the 9C8 monoclonal antibody, as previously described (8).

cDNA Representational Difference Analysis (RDA), Cloning of DOL54, and Analysis of Its Expression. Poly(A)$^+$ RNA and cDNA was prepared from Cre-transduced *Tls.m2* cells, Cre-transduced wild-type cells, and nontransduced *Tls.m2* cells. The former served as the tester population, whereas the latter two constituted the driver. RDA was performed as previously described (12, 35), and the DpnII fragment corresponding to the region between nucleotides 3,384 and 3,693 of DOL54/megakaryocyte stimulatory factor (MSF) (accession no. HSU70316) was subcloned and sequenced. Northern blot analysis of DOL54 mRNA was performed on total cellular RNA (30 μg/lane) by using the insert obtained by RDA or the full length human cDNA. The recombinant retroviruses used to transduce TLS-CHOP and its various derivatives and mutants have been previously described (6). TLS-C/EBPβ is a fusion of human TLS and murine IL6-DBP at amino acid residues 275 and 31, respectively. TLS-CREB is a fusion at residues 275 and 67, respectively. MEFs with defined *C/ebpβ* genotypes were produced from embryos derived by crossing the *Tls.m2* strain described above to a strain carrying the *C/ebpβ−* allele (36) and backcrossing the compound heterozygotes to the *C/ebpβ−* parent. Polyclonal antiserum to DOL54/MSF was produced by immunizing rabbits with recombinant peptide expressed in Chinese hamster ovary (CHO) cells (residues 1–810 of the human MSF). The antiserum was used in immunocytochemistry at a dilution of 1:500. Tumorigenicity of CHO cells expressing full length DOL54/MSF was tested by injecting 10^6 cells into each of three subcutaneous sites in nude mice. Differentiation of 3T3-L1 cells to adipocytes was promoted as previously described (8), whereas MEFs were exposed to 5 μM BRL48482 in regular growth media.

RESULTS

To isolate genes *do*wnstream *o*f the *l*iposarcoma-associated fusion oncoproteins (DOLs), we compared, by means of a RDA, the pattern of mRNAs expressed in otherwise isogenic cells that do and do not express TLS-CHOP. Early attempts to perform this analysis by using a retrovirus that encodes TLS-CHOP or EWS-CHOP were frustrated by the frequent isolation of retrovirally encoded genes. To circumvent this difficulty, we engineered a mouse strain that carries a conditional TLS-CHOP-expressing germline allele of *Tls* and analyzed cells in which this allele was conditionally activated. The modified allele was created by homologous recombination at the *Tls* locus. The *Tls* coding region was interrupted in embryonic stem cells by a cassette that contained a *Neo* structural gene flanked by *loxP* sites and followed by the *CHOP* coding sequence and suitable termination signals. Before Cre-induced recombination at the *loxP* sites, the allele (referred to as *Tls.m2*) is carried in the germline of mice as a hypomorphic mutant of *Tls*, encoding a TLS-Neo fusion protein. After site-specific recombination, the allele encodes a TLS-CHOP fusion protein that is essentially identical to authentic TLS-CHOP (Fig. 1 *A* and *B*).

MEFs derived from *Tls.m2* embryos or wild-type isogenic sibling embryos were infected with a Cre-expressing retrovirus. Retroviral infection leads to high-level expression of TLS-CHOP detected by Western blotting (Fig. 1*C*) or immu-

nohistochemistry (Fig. 2*B*, micrograph 1). In keeping with previous experiments in which TLS-CHOP's transforming capability was found to be limited to immortalized cells (6, 20), expression of TLS-CHOP had no detectable effect on these primary cells. RDA was used to identify genes expressed in the Cre-transduced *Tls.m2* cells but not in Cre-transduced wild-type cells or in parental *Tls.m2* cells (inclusion of the latter cDNA in the driver population eliminated the *CHOP* fragments from the represented pool). This procedure led to the identification of two genes that were differentially expressed in the TLS-CHOP$^+$ cells. The first was identical to *DOC4*, a CHOP-dependent stress-induced gene (12). It was expressed only at low levels in the TLS-CHOP$^+$ cells and was not evaluated further. The second gene, *DOL54*, was expressed at high level in the TLS-CHOP$^+$ cells, but not in the parental *Tls.m2* cells (Fig. 2*A*).

Next we examined the ability of various derivatives of TLS-CHOP, introduced by retroviral transduction, to activate *DOL54* expression in MEFs. Both TLS-CHOP (Fig. 2*B*, lanes 1 and 2) and EWS-CHOP (lane 8) activated the endogenous *DOL54* gene. Overexpression of germline CHOP only minimally induced *DOL54* (lane 9). Removal of the CHOP leucine-zipper dimerization domain or the DNA-binding basic region from TLS-CHOP abolished all expression of *DOL54* (lanes 3 and 4). Nononcogenic derivatives (6), in which CHOP was fused to potent heterologous activation domains of either VP-16 or C/EBPα, were without effect (lanes 5 and 6). The difference in the ability of CHOP and the nononcogenic derivatives to activate DOL54 may be caused by slightly lower levels in expression of the latter. EWS-FLI1, a fusion oncoprotein that shares the N-terminal domain of EWS-CHOP but contains an unrelated DNA-binding domain of the Ets-family type (37), was likewise inactive (lane 7). EWS-FLI1 serves as an important control because it dissociates activation of *DOL54* from transformation, a point that is also supported by the inability of oncogenic *Ras* to activate *DOL54* (data not shown). These experiments indicate that features of TLS-CHOP that are necessary for transformation are also required for activation of *DOL54* expression, but that transforming oncogenes that operate by different pathways do not activate the gene.

In MEFs, the major dimerization partner of CHOP and TLS-CHOP is C/EBPβ (6, 12, 38). We reasoned that if TLS-CHOP activates *DOL54* by forming a productive dimer with a C/EBP partner, MEFs genetically deficient in C/EBPβ would be impaired in *DOL54* expression. If, on the other hand, *DOL54* were activated through a mechanism that involves inhibition of C/EBP binding to certain target sequences by TLS-CHOP, absence of C/EBPβ would not impair *DOL54* expression. The *Tls.m2* allele was therefore introduced into the *C/ebpβ−* background by mating of mutant mice, and the expression of *DOL54* in response to Cre infection of MEFs was compared between cells with *C/ebp$\beta\pm$;Tls.m2/+* and *C/ebpβ−/−;Tls.m2/+* genotypes. Fig. 3*A* shows that lack of C/EBPβ abolished activation *DOL54* by TLS-CHOP. To

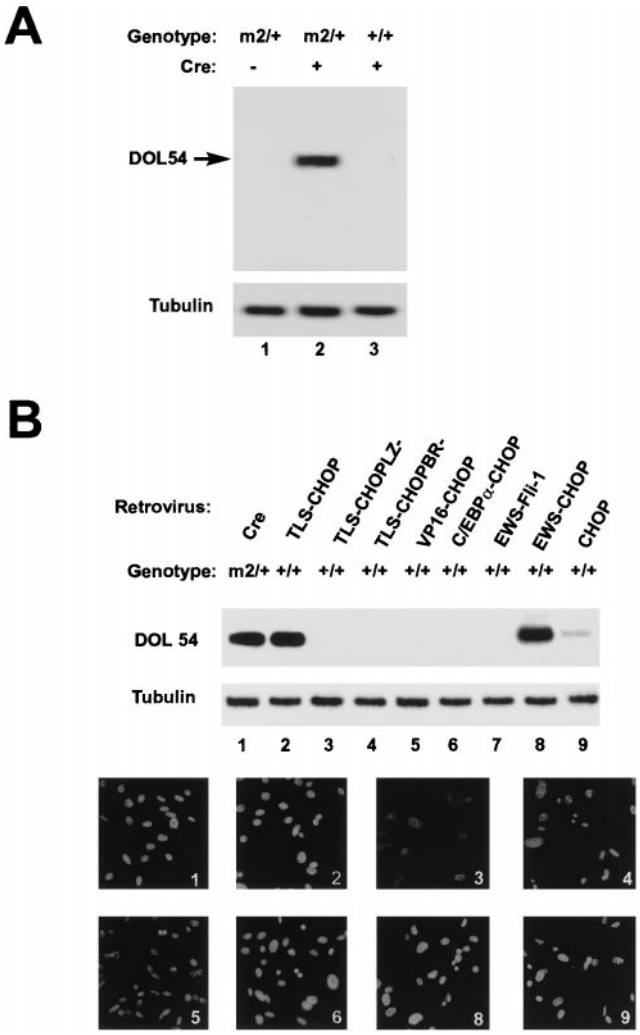

FIG. 2. DOL54 expression is induced by *TLS-CHOP*. (*A*) Northern blot of total RNA from the MEFs shown in Fig. 1*C* hybridized to the labeled DOL54 cDNA. (*B*) Northern blot of RNA from wild-type MEFs transduced with retroviruses expressing the indicated proteins. LZ$^-$ and BR$^-$ represent deletions of the leucine-zipper dimerization domain and basic-region DNA-binding domains of TLS-CHOP, respectively. VP16-CHOP and C/EBPα-CHOP are fusions of CHOP to the activation domain of herpes-virus VP16 and rat C/EBPα proteins, respectively. The photomicrographs (*Lower*) are from fixed samples of the transduced cells stained with antiserum to CHOP, serving as a control for the expression of the various fusion proteins.

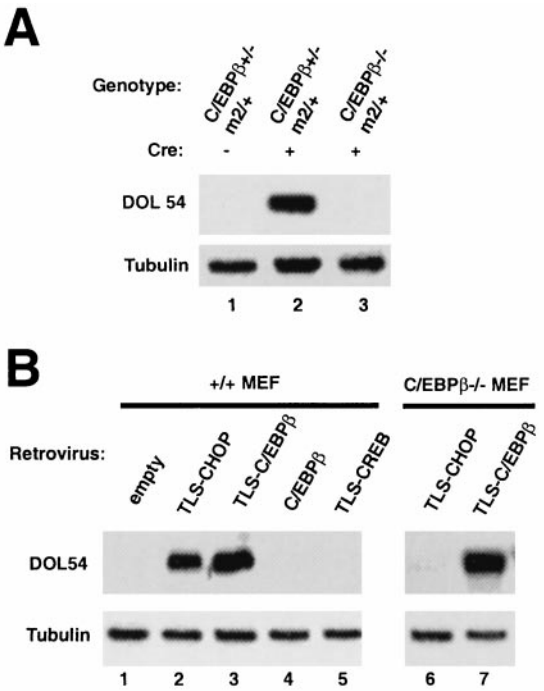

FIG. 3. Activation of *DOL54* by *TLS-CHOP* requires a dimerization partner. (*A*) Northern blot analysis of MEFs with the mutant *Tls* allele and having (+/−) or lacking (−/−) a functional C/ebpβ allele. The blot was hybridized with the DOL54 cDNA insert (*Upper*) or tubulin as loading control (*Lower*). (*B*) DOL54 Northern blot of wild-type (+/+) or C/ebpβ−/− MEFs transduced with retroviruses expressing the indicated proteins.

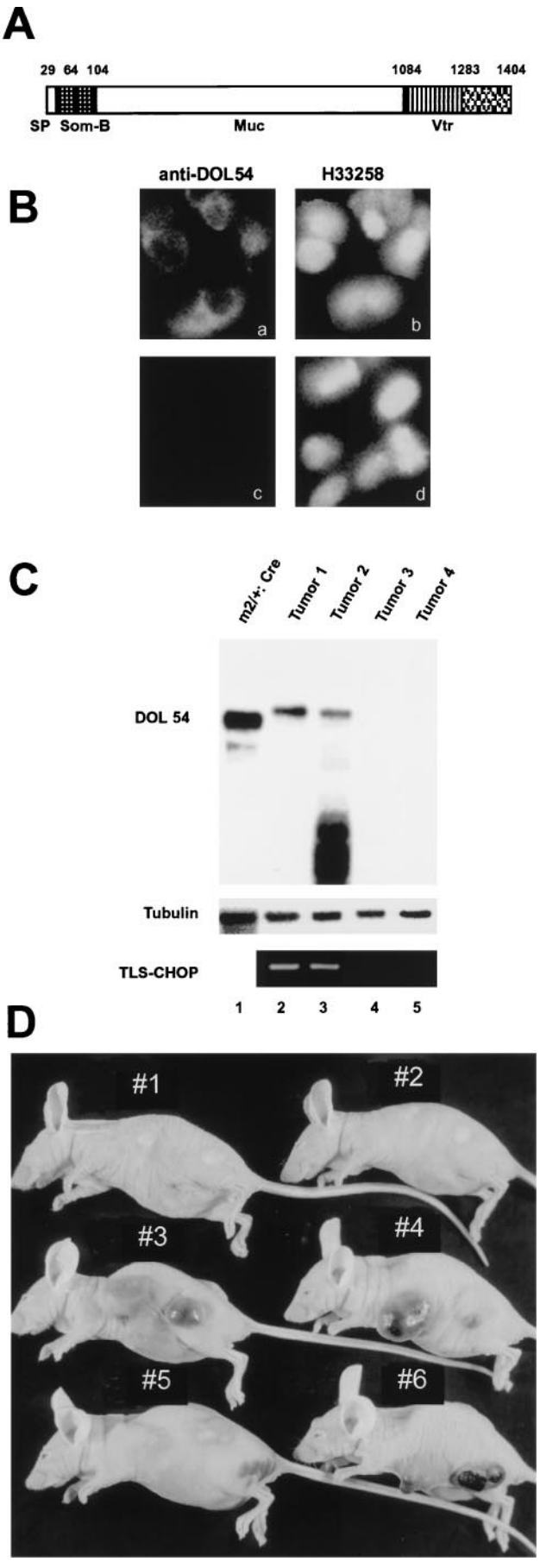

FIG. 4. (*A*) Schematic diagram of the predicted peptide sequence of DOL54. The signal peptide (SP), two somatomedin B-like repeats (Som-B), mucin-homology domain (Muc), and vitronectin-related sequences (Vtr) are indicated. (*B*) DOL54 immunostaining of fixed but nonpermeabilized CHO cells. The cells (*Top*) were stably transfected with a full length DOL54 expression plasmid, whereas the cells on the bottom are the parental strain. (*C*) Northern blot of RNA from human tumor samples hybridized to a DOL54 probe and tubulin loading control. The lowest gel is a human TLS-CHOP reverse transcription–PCR analysis on the same RNA. Note that the tumors positive for TLS-CHOP were also positive for DOL54. The *TLS-CHOP* mRNA in the positive control MEFs (lane 1) is a hybrid of murine *Tls* and human *CHOP* and is not amplified by the PCR primers used here. (*D*) Tumorigenicity of individual CHO clones expressing DOL54 (mice 3–6) and parental cells that do not express the protein (mice 1 and 2) injected into nude mice and photographed 10 days after injection of 10^6 cells into each of three subcutaneous sites. The CHO clone injected into mouse no. 5 expressed ≈3-fold lower levels of DOL54 mRNA than the clones injected into mice 3, 4, and 6).

determine whether C/EBPβ exerts its essential role in *DOL54* expression as a dimerization partner of TLS-CHOP or whether it acts in an essential parallel pathway, we exploited an artificial derivative of TLS-CHOP, TLS-C/EBPβ, in which the DNA-binding and dimerization domain of CHOP is replaced by that of C/EBPβ: In contrast to CHOP (and TLS-CHOP) that are incapable of homodimerization and are dependent on a dimerization partner to activate downstream target genes (11, 12), a fusion between TLS and the DNA-binding and dimerization domain of C/EBPβ is predicted to bind DNA as a homodimer. To the extent that C/EBP dimers can also bind some C/EBP-CHOP binding sites (11, 13), we considered it possible that TLS-C/EBPβ might be able to induce *DOL54* in *C/ebp*$\beta-/-$ cells. Expression of TLS-C/EBPβ potently activated *DOL54* expression in both wild-type and *C/ebp*$\beta-/-$ MEFs (Fig. 3*B,* lanes 3 and 7). Overexpression of either wild-type C/EBPβ or TLS-CREB, a different artificial chimera that contains the DNA-binding and homodimerization domain from the related B-Zip protein CREB, was without effect (lanes 4 and 5). These findings are most consistent with a model whereby activation of *DOL54* by TLS-CHOP requires the formation of a productive dimer with C/EBPβ to activate a direct target gene. The latter could be *DOL54* itself or an upstream intermediate.

Sequencing of the DOL54 insert revealed it to be the murine homologue of a human gene initially characterized as MSF. The latter assignment was based on the observation that a processed N-terminal peptide derived from the full length secreted protein was found in the urine of certain thrombocytopenic patients and exerts a stimulatory effect on maturation of megakaryocytic progenitors (K.J.T. *et al.*, unpublished results). The full length human DOL54/MSF is a protein of 1,404 residues with an N-terminal signal peptide followed by regions with similarity to somatomedin-B, various mucins, and vitronectin, all of which are found in secreted matrix-associated proteins (Fig. 4*A*). Staining of fixed nonpermeabilized CHO cells expressing human DOL54/MSF protein with a specific antiserum revealed the presence of the antigen on the cell surface (Fig. 4*B*). Immunoprecipitation experiments revealed the absence of soluble protein in the culture supernatant (not shown). Together these findings suggest that secreted DOL54/MSF associates with the cell surface or the insoluble extracellular matrix.

Expression of DOL54 was evaluated in human liposarcomas expressing *TLS-CHOP*. Northern blot analysis revealed high levels of DOL54 mRNA in TLS-CHOP$^+$ tumors but not in mesenchymal tumors that are not expressing TLS-CHOP (Fig. 4C). These findings were supported by immunostaining of human tumors (data not shown). To determine whether expression of DOL54 might play a role in tumor development, we compared the ability of DOL54-expressing CHO cells and parental CHO cells to grow as tumors in nude mice. Cells from three independently derived DOL54-expressing clones pro-

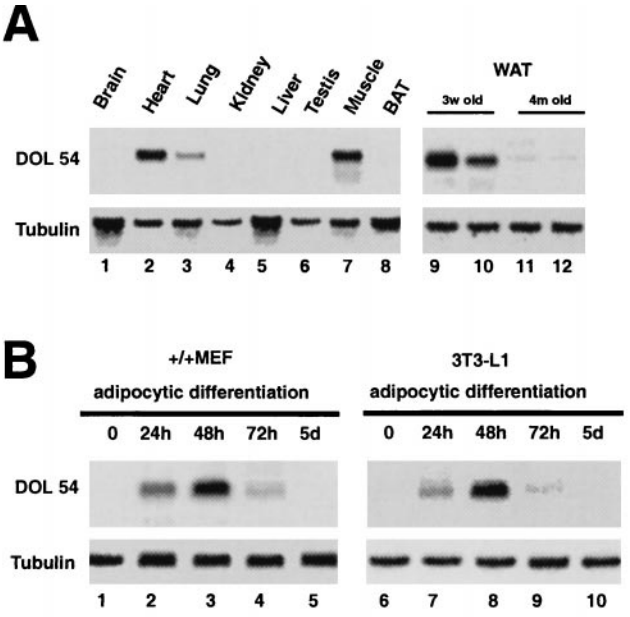

FIG. 5. Northern blot analysis of DOL54 expression in mouse tissues and during adipogenesis. (*A*) Total RNA from the indicated organs of adult mice (*Left*) or white adipose tissue of young (3 wk) and adult mice (4 mo) (*Right*) were sequentially hybridized with the DOL54 cDNA and tubulin. (*B*) Total RNA from MEFs or 3T3-L1 cells differentiating *in vitro* to adipocytes. Cells were harvested at the indicated time points after being exposed to an *in vitro* differentiation protocol.

duced large hemorrhagic tumors when injected subcutaneously into nude mice (Fig. 4*D*, mice nos. 3, 4, and 6). Parental cells that express no DOL54 gave rise to smaller tumors that were nonhemorrhagic (mice nos. 1 and 2). A CHO clone expressing low levels of DOL54 gave an intermediate phenotype (mouse no. 5). These results suggest that DOL54 may play a role in the tumorigenicity of TLS-CHOP positive cells.

Northern blot analysis of normal mouse tissues revealed DOL54 mRNA in heart, muscle, and lung, but most notably in white adipose tissue of young but not older animals (Fig. 5*A*). The association of DOL54 with ongoing adipogenesis is further strengthened by the observation that its expression is induced both in adipogenic 3T3-L1 cells and in MEFs by treatments that promote adipocytic differentiation (Fig. 5*B*). Induction occurs early in the process of differentiation and peaks at 48 hr, whereas markers of the mature adipocytic phenotype are usually observed only after 72 hr (8). These findings suggest that the presence of TLS-CHOP in MEFs, a pool of mesenchymal precursors, promotes the unscheduled expression of a gene that is expressed transiently during normal adipocytic differentiation.

DISCUSSION

This study documents an epistatic relationship between *TLS-CHOP*, an oncogene frequently found in human liposarcoma, and *DOL54/MSF*, a gene encoding a secreted protein. This relationship is of interest because of the clues it provides into TLS-CHOP action at both the mechanistic and physiological levels. The requirements for *DOL54* activation include intact CHOP DNA-binding and dimerization domains and the presence of a suitable dimerization partner. These findings strongly support a model whereby TLS-CHOP forms a productive heterodimer with a C/EBP partner (most likely C/EBPβ), and this dimer interacts with a target gene that may be DOL54 itself or a gene upstream of DOL54. Three other genes known to lie downstream of *CHOP*: DOC1, DOC4, and DOC6, are also activated by *TLS-CHOP* (12, 13). However, their level of activation by the oncoprotein is not measurably different from their activation by CHOP (data not shown). Moreover, activation of the DOCs by both CHOP and TLS-CHOP is stress-dependent, indicating that the presence of a TLS-type activation domain does not abrogate the requirement for second signal for their induction (12, 13). DOL54 is the first example of a cellular gene preferentially activated by TLS-CHOP. Activation is constitutive and depends on the special attributes of the N terminus of TLS or EWS; these cannot be substituted by heterologous activation domains. The relationship between *TLS-CHOP* and *DOL54* indicates that the functional distinction between the oncogene and the normal cellular gene, previously noted in terms of their different transforming activities (6), is also reflected in their differential ability to activate downstream targets.

The DNA-binding activity of CHOP and TLS-CHOP is entirely dependent on the formation of a complex with a C/EBP dimerization partner (11). The requirement for C/EBPβ in *DOL54* activation by TLS-CHOP is thus easy to rationalize in these terms. DNase I footprint analysis and methylation interference assays indicate that in such heteromeric complexes, it is the C/EBP partner that provides most of the sequence-specific DNA-binding activity, with the CHOP component playing an ancillary role. C/EBP dimers can even bind to known CHOP-C/EBP target sequences, albeit with reduced affinity (11, 13). The ability of the artificial TLS-C/EBPβ chimera to activate *DOL54* in *C/ebp*$\beta-/-$ cells presumably reflects such binding either to a target sequence in *DOL54* itself or in an upstream activator of *DOL54*. Overexpression of C/EBPβ or CHOP does not fully activate *DOL54*, providing further evidence for the essential role played by the TLS N-terminal domain. These results are consistent with a model whereby the activation domain of TLS provides an essential function that is revealed in the context of the relevant target gene's promoter. However, because the TLS-C/EBPβ chimera also contains most of the C/EBPβ peptide sequence, we cannot exclude a role for the transactivation domain of TLS-CHOP's dimerization partner in DOL54 expression. It is also formally possible that the presence of the TLS N terminus influences the DNA-binding properties of the TLS-CHOP chimera and that the specific action of the oncoprotein is mediated at that level. To distinguish between these possibilities, one would need to identify the cis-acting target sequences that are relevant to the activation of *DOL54* by the oncoprotein.

The physiological role of *DOL54/MSF* is not known. The gene encodes a secreted polypeptide that associates, at least in part, with the insoluble extracellular matrix or plasma membrane. CHO cells expressing DOL54 grow faster as tumors than parental nonexpressing CHO cells when the two are injected into nude mice. *DOL54* is induced transiently during differentiation of adipogenic precursors *in vitro* and is expressed normally in the white adipose tissue of young mice (Fig. 5). Adipocytic differentiation is associated with significant alterations in the composition of the extracellular matrix. These are thought to feed back on the differentiating cells to effect changes such as a decrease in actin biosynthesis (39–41). It is tempting to speculate that secretion of DOL54 may play a role in defining certain functional properties of the extracellular matrix and thus in regulating aspects of cellular phenotype during adipocytic differentiation. In this model, misexpression of DOL54 by TLS-CHOP would play a role in oncogenesis through the unscheduled induction of a signal that is normally provided only in the context of the highly regulated process of differentiation. DOL54 expression is induced early in adipocytic differentiation (Fig. 5*B*), at a time when the various C/EBP isoforms are actively promoting that process (16, 19). The demonstrated ability of TLS-CHOP to inhibit the activity of C/EBP proteins (10, 20) might be part of the

abnormal context in which TLS-CHOP induces *DOL54* expression in tumor precursors.

Liposarcomas associated with expression of TLS-CHOP frequently have a myxoid appearance (1). This is because of the deposition of large quantities of extracellular matrix. Deposition of DOL54 protein in the vicinity of the neoplastic component of such tumors may play a role in the development of this phenotype. Expression of DOL54 is clearly not sufficient for the development of a myxoid stroma, since the tumors that arose in the nude mice injected with CHO cells secreting the protein had very little extracellular matrix deposition in tumor stroma. TLS-CHOP is not uniformly associated with expression of *DOL54*. Rather the link between the two is limited to primary cells and tumor samples. For example, expression of TLS-CHOP in immortalized rodent fibroblasts does not induce the endogenous *DOL54* gene and cell lines derived from SV-40 immortalized human liposarcoma cells, which stably express TLS-CHOP, do not express *DOL54* (data not shown). Cellular context thus plays an essential permissive role in the ability of TLS-CHOP to activate *DOL54*. The nature of the cellular precursor of human liposarcomas is not known. To the extent that it is capable of differentiation to an adipocytic cell, it is likely to be a mesenchymal precursor of fat. The primary MEFs used in our screen to isolate *DOL54* represent a collection of different cell types and are likely to include such precursors. These considerations, in conjunction with the fact that *DOL54* is expressed in the tumors, underscores the potential relevance of relationship between *DOL54* and tumorigenesis.

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