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Overexpression of a member of the pentraxin family (PTX3) in human soft tissue liposarcoma ☆

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ABSTRACT

A unique feature of human soft tissue liposarcoma is a stable (12;16)(q13;p11) translocation observed mainly in myxoid and roundcell liposarcomas. This translocation results in FUS/CHOP fusion transcripts with a corresponding oncogenic protein. We hypothesised that genes downstream of FUS/CHOP might serve as attractive candidates for novel tumour associated antigens. Among a panel of analysed genes, only pentraxin related gene (PTX3) demonstrated high expression in liposarcomas as compared to normal tissues. The analysis of RNA and protein expression demonstrated concordant results. However, the level of RNA and protein overexpression did not correlate in all cases. Finally, PTX3 expression was not related to presence of a FUS/CHOP fusion transcript within the liposarcoma tissues.

PTX3 has been associated with adipocyte differentiation and now, additionally, is characterised by a markedly increased expression in human soft tissue liposarcoma. This finding mandates further research efforts to clarify the exact role of PTX3 in liposarcoma oncogenesis.

1. Introduction

Soft tissue liposarcoma is the most frequently diagnosed sarcoma in adulthood. Clinically, these tumours present with a wide pattern of malignant potential, ranging from tumours remaining local up to aggressive tumours with difficulties achieving local tumour control and resulting distant metastasis. In particular, cases diagnosed with distant metastases pose a frustrating therapeutic challenge, since this tumour frequently resists chemotherapy. In this setting, identifying tumour-associated antigens is a central prerequisite for developing alternative drugs affecting tumour growth.

Myxoid and roundcell subtypes of liposarcoma demonstrate a (12;16)(q13;p11) chromosomal translocation in most analysed cases. While a small subset of tumours is affected by a (12;22) translocation, frequently the (12;16) translocation

☆ Dedicated to Hubert Szelenyi, a young hematologist and oncologist who died too early, commemorating his contribution to oncology.

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results in the formation of a FUS/CHOP fusion gene. Herein, the CHOP gene comes under the control of the FUS gene promoter. The control of adipocyte differentiation and growth arrest as mediated by normal CHOP gene expression[1,2] is lost due to the fusion. As a result, malignant transformation of NIH 3T3 and ST-13 cells have been observed in transfection experiments.[3–5] In addition, the permanent overexpression of the FUS/CHOP protein in transgenic mice leads to tumour development in the brown fat mimicking human soft tissue liposarcoma.[6] While convincing evidence of the function of FUS/CHOP as an oncogene has been collected using different analytic approaches, little is known about how the fusion gene interferes with adipocyte differentiation, ultimately leading to the formation of liposarcoma.

Our group was able to identify five genes demonstrating differential expression after FUS/CHOP transfection experiments with NIH 3T3 cells. Array results were confirmed by Northern blot and yielded the following genes: pentraxin related gene (PTX3) [syn.TSG14], osteoblast-specific factor 2 (osf-2), leukoprotease inhibitor (SLPI), basic Kruppel-like factor (bklf), cyclophilin B (cyp B) and connective tissue growth factor related protein 2 (WISP 2).[5] None of these genes have been associated with liposarcoma development to date or have been associated with either one of the fusion gene partners.

In order to delineate gene expression controlled by FUS/CHOP, we analysed the specific role of candidate proteins in human liposarcoma specimen.

2. Patients and methods

2.1. Patients

Tissue samples were obtained from 29 patients treated for liposarcoma between 1990 and 2003 at the Department of Surgery, University of Heidelberg, and the Surgical Department of the University Clinic Mannheim. Approval of both local ethic committees was obtained. The histological sections were examined by an expert pathologist and immunohistochemistries were classified by two independent examiners and reviewed by one of the authors (R.G.). Soft tissue sarcomas were classified according to Fletcher, Krishnan Unni and Mertens.[7]

The study cohort included 29 patients (20 male, 9 female) with a median age of 53 years (range: 31–84 years). Among these patients, three developed one recurrence and one developed three recurrences, all of which were re-resected. Therefore, 35 tumour samples of 29 liposarcoma patients were analysed. The patients' characteristics and their corresponding tumours are found in Table 1.

2.2. Tissue samples

Tumour and normal tissue collection were performed as previously described.[8] The cell lines 1955–91 (FUS/CHOP positive human liposarcoma) and SW 872 (FUS/CHOP negative human liposarcoma) served as positive and negative controls. 42 samples of normal tissues (skin, muscle, colon, kidney, stomach, pancreas) and 19 non-lipomatous soft tissue sarcoma tumours (vascular, smooth muscle, chondro-osseous, fibroblastic, fibrohistiocytic and uncertain differentiation origin) served as controls.

2.3. RNA extraction and FUS/CHOP nested RT-PCR

RNA was extracted from normal and tumour tissue using the mirrorblock technique of cryocut material to ensure representative tissues in the RNA extraction samples. Processing of RNA and technical details of the RT-PCR system has been described before.[8] The PCR reactions were repeated at least twice from independent RNA samples in two different laboratories, and negative and positive controls were included in all reactions. Amplified PCR-products were visualised under UV light (280 nm wave length) after electrophoresis in 1.3% agarose gels stained with ethidium bromide (0.5 μg/ml). To detect the specific fusion transcript of FUS/CHOP, a sequence analysis of RT-PCR products was performed as described before.[8]

2.4. Relative quantitative real time RT-PCR

All olinucleotides were designed with the PRIMER 3 software.[9] The PTX3 transcript was amplified with PTX3_1_f (seq. 5′/3′: TCTCTGGTCTGCAGTGTTGG) and PTX3_1_r (seq. 5′/3′: TGAAGAGCTTGTCCCATTCC) generating a 152 bp fragment. The constitutively expressed gene HMBS (Hydroxymethylbilane synthase) was chosen as the housekeeping gene[10] and a 151 bp fragment was amplified using primers HMBS_f (seq. 5′/3′: CCAGGACATCTTGGATCTGG) and HMBS_r (seq. 5′/3′: CAGGTACAGTTGCCCATCCT). All Primers were spanning exon/exon boundaries to avoid amplification of contaminating genomic DNA. PCR was conducted with 20 μl reaction volumes of 10 μl SYBR®Green PCR master mix (QuantiTec SYBR, Qiagen, Hilden, Germany), 2 μl cDNA, 2 μl of each primer [10 μM] and 4 μl of water. The thermal cycling conditions were comprised of an initial denaturation step at 95 °C for 15 min and 45 cycles at 95 °C for 15 s, 55 °C for 20 s and terminal elongation at 72 °C for 10 min. All PCR reactions were performed using a LightCycler (Roche). Specificity of amplified products was checked by melting curve analysis and agarose gelelectrophoresis with ethidiumbromide staining and only primer-dimer- and artefact-free reactions were considered valid. Negative RT-PCR samples, which were generated exactly in parallel to cDNA synthesis solely without adding reverse transcriptase, were investigated independently to exclude genomic DNA contamination. Data analysis was carried out using the LightCycler software (version 3.5, Roche). Each data point represents the results of duplicate experiments. The relative amount of transcript in any sample was calculated using the comparative delta Ct-Method as previously described.[11]

2.5. Western blot

Proteins from tumour tissue, normal tissue and cell lines were solubilised in Laemmli buffer. The proteins then were electrophoresed in 10% SDS gels and blotted onto PVDF membranes (Biorad, München,Germany). Filters were probed for the following antibodies: PEBP2aA (=OSF2) [sc 8566 Santa Cruz]; PTX3 [supplied by Mantovani A. & Peri S. Department of

Table 1 – Summary of patients with liposarcoma, their respective tumour characteristics and the results of the analysis of PTX3 expression

No.	Sex	Age	Histology	Grading	FC-status	REF PTX3	N IHC PTX3	T IHC PTX3	N WB PTX3	T WB PTX3
1a	f	34	pleomorph	high	positive	0,23	0	4	0	4
2	m	70	well diff.	low	negative	0,89	0	0	0	2
3	m	37	myxoid	low	negative	1,31	0	2	0	1
4	m	53	dediff.	high	negative	1,39	2	3	2	3
5	m	64	myxoid	low	negative	2,2	2	3	3	1
1b	f	37	pleomorph	high	positive	2,66	0	3	0	3
6	m	66	well diff.	low	negative	3,2	3	2	2	2
7	m	74	pleomorph	high	negative	4,32	0	3	0	2
8	m	31	myxoid	low	positive	6,45	0	2	2	3
9	f	60	well diff.	low	negative	6,89	1	3	0	4
10	m	41	myxoid	high	negative	7,06	1	1	3	3
11	m	59	myxoid	high	negative	7,19	1	1	2	2
12a	f	53	myxoid	high	positive	9,88	0	2	1	2
13	m	49	myxoid	high	positive	15,62	0	1	1	2
14a	f	66	myxoid	high	negative	17,63	0	3	2	2
12b	f	50	myxoid	high	positive	19,43	1	4	3	3
15	f	77	well diff.	low	negative	35,38	2	3	0	0
16	m	78	pleomorph	high	negative	37,79	2	2	2	3
14b	f	65	myxoid	high	negative	48,5	0	4	0	2
17	m	38	myxoid	high	positive	48,84	0	3	1	3
18	f	58	well diff.	low	negative	84,04	1	3	0	2
19	m	37	myxoid	low	positive	92,09	0	1	1	4
20	f	55	myxoid	low	positive	115,36	1	2	0	2
21	m	34	myxoid	low	positive	138,62	0	2	2	3
22	m	84	dediff.	low	negative	143,59	3	3	0	0
23	m	61	dediff.	high	negative	268,73	0	2	0	1
24	m	54	myxoid	high	positive	285,04	0	0	0	3
25a	f	45	roundcell	high	positive	307,62	n.d.	4	0	3
26	m	58	well diff.	high	negative	362,04	3	1	0	4
27	m	42	pleomorph	high	negative	385,34	0	4	1	4
25b	f	47	roundcell	high	positive	435,04	0	2	1	3
25c	f	51	roundcell	high	positive	464,65	n.d.	2	n.d.	2
25d	f	44	roundcell	high	positive	654,84	3	4	3	4
28	f	41	roundcell	low	positive	n.d.	1	3	0	2
29	m	63	dediff.	low	negative	n.d.	0	1	1	1

Patients sorted with increasing PTX3 expression in real time RT-PCR, therefore corresponding to Fig. 2.
Abbrev.: *m* = male; *f* = female; histology = subtype of liposarcoma (well diff. = well differentiated; dediff. = dedifferentiated); FC-status = result of FUS-CHOP RT-PCR analysis; REF = relative expression fold, logarithmic increase of the PTX3 expression comparing tumour and normal tissue samples; PTX3 = pentraxin related gene; IHC = immunohistochemistry; WB = Western blot; n.d. = not determined.

Immunology & Cell Biology, Istituto di Ricerche Farmacologiche, Milano] SLPI [sc-10534 Santa Cruz]; WISP-2 [sc-8867 Santa Cruz].The resulting bands were visualised with chemiluminescence (Perkin Elmer, Boston, USA).

2.6. *Immunohistochemistry*

Cryo sections of tumour tissue and normal tissue were subjected to peroxidase blockade (H_2O_2 3%). A serum blockade was followed by incubation with the first and second antibody, the latter biotinilated. After application of the avidin-biotin complex, immunostaining was visualised by the peroxidase reaction (ABC-method). Respective antibodies analysed are identical to the ones utilised in Western blot. Control sections included positive and negative controls. The analysis of the stained specimen with the different antibodies was performed by independent evaluations of two of the authors (A.A. & F.W.) and controlled by a third author (R.G.). A semiquantitative score for positive/negative staining of cells and cytoplasm was defined as the following: 0 = absence of immunoreactive cells/cytoplasm; 1 = 1–10% of cells/cytoplasm stained; 2 = 11–25% of cells/cytoplasm stained; 3 = 26–50% of cells/cytoplasm stained; 4 = >50% of cells/cytoplasm stained.[12]

2.7. *Statistical analysis*

SAS software (release 8.0, SAS Institute, Inc. Cary, NC) was used for statistical analysis. Potential relationships between defined histologies, FUS-CHOP status and protein expression were examined with the Fisher's exact test. Statistical significance was assumed at $p < 0.05$. Correlations between the logarithmic variable ΔCT and different characteristics of the patients and their tumours were evaluated by unpaired Student's *t*-test. Potential correlations of ΔCT with subsets of tumour histology were evaluated with the Dunnett test. Different expression profiles of PTX3 in tumour and normal tissue were analysed by the test of symmetry.

3. Results

3.1. *Distribution of FUS/CHOP fusion transcript in the analysed liposarcomas*

With all subtypes of liposarcoma represented, the myxoid subtype was predominant (43%), and FUS/CHOP was detected in 60% of these samples. The analysed five roundcell liposarcoma samples, at present categorised as a subset of myxoid liposarcoma, all tested positive for the FUS-CHOP fusion transcript. Beside myxoid and roundcell liposarcomas, only one patient with a pleomorphic liposarcoma, later developing a local recurrence, tested positive for FUS-CHOP fusion transcript. All positive samples were subjected to DNA sequencing to confirm the specificity of the visualised bands. Well-differentiated and dedifferentiated liposarcomas all tested negative for FUS-CHOP. All observed tumour recurrences from individual patients (e.g. primary tumour, local recurrence and/or distant metastases) either proved FUS/CHOP negative or FUS/CHOP positive (Table 1).

3.2. *Immunohistochemistry of genes with differential expression in cDNA array experiments*

In FUS/CHOP transfection experiments, we found a considerable number of genes overexpressed in FUS/CHOP expressing NIH3T3 cells. Therefore, we evaluated the expression of these genes in human liposarcomas in a pilot study by means of immunohistochemistry and were unable to find significant differences among the following genes: SLPI, osf-2, WISP-2, JunB, adrenomedullin, Cyclin D2, phospholipid transfer protein, Cyp1-b-1, decay accelerating factor 1 and 2, homeodomain-containing transcription factor gene, small inducible cytokine subfamily D, latexin, CD24a antigen and BKLF. A subset of these results is given in Table 2. Only PTX3 demonstrated a distinct expression enhancement in the respective tumour tissues as compared to other genes ($p < 0.001$). Surprisingly, PTX3 also showed an increased expression in non-lipomatous soft tissue sarcomas (NLSTS) ($p < 0.001$) in immunohistochemistry analysis. Regarding an overexpression of PTX3, no statistical difference between liposarcomas and NLSTS was observed ($p = 1.0$).

3.3. *Real time PCR of PTX3 transcripts demonstrates a distinct overexpression in liposarcoma*

To confirm these preliminary data, we examined the expression of PTX3 transcripts by real time RT-PCR in 33 liposarcoma samples and six NLSTS. The ΔCT values of liposarcomas and NLSTS were compared to the pooled values of 42 normal tissues. No correlations of the PTX3 expression was demonstrated for age, sex, or grading of the liposarcoma patients. However, in general, we found a striking higher expression of PTX3 in the liposarcoma samples as compared to normal tissue (Fig. 1). While 61% of the liposarcomas revealed more than a 10-fold increase in PTX3 expression, only 12% demonstrated a low expression comparable to the pooled normal tissue. 27% of the tumours demonstrated a 2–9 fold increase of PTX3 expression. This increase of expression was irrespective of the FUS/CHOP fusion transcript status of the tested tumour ($p = 0.17$). However, among histological subtypes, roundcell liposarcomas presented with significantly higher PTX3

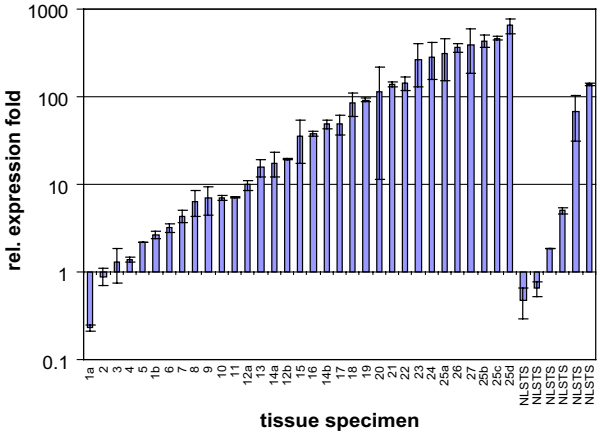

Fig. 1 – Quantitative RT-PCR analysis of PTX3 expression in liposarcomas and non-lipomatous soft tissue sarcomas (NLSTS). The relative expression fold is the logarithmic increase of PTX3 expression in comparison to a pooled normal tissue sample. Tissue specimen enumeration corresponds to the patients data in Table 1.

Table 2 – Results of immunohistochemistry in a subset of tumours searching for proteins selectively overexpressed in liposarcoma

Protein	Immunohistochemistry in liposarcoma		Immunohistochemistry in non-lipomatous STS	
	positive	negative	positive	negative
PEBP2aA	0	7	0	7
PTX3	27 (77%)	8 (23%)	15 (79%)	4 (21%)
SLPI	0	10	0	5
WISP2	1	9	0	5

Significant overexpression of PTX3 as compared to other proteins in liposarcomas ($p < 0{,}001$). Significant overexpression of PTX3 in non-lipomatous soft tissue sarcomas ($p < 0{,}001$).

Abbrev.: LS = liposarcoma; STS = soft tissue sarcomas; PEBP2aA = OSF2 = osteoblast specific factor 2 gene; PTX3 = pentraxin related gene; SLPI = secretory leukoprotease inhibitor gene; WISP2 = connective tissue growth factor related protein 2.

expression as compared to all other liposarcoma subtypes (significant by Dunnett test with $\alpha = 0.05$).

3.4. *Increased expression of PTX3 in soft tissue liposarcoma demonstrated by immunohistochemistry*

After focussing on PTX3, we performed a systematic evaluation of normal and tumour tissue utilising immunohistochemistry. Examples of the stained tissues can be seen in Fig. 2. Since liposarcoma specimen from different anatomical localisations were evaluated, different normal tissues appeared as resection margins of the respective patients. While negative staining of the cell nucleus was always observed in fat tissue, for example normal colon and kidney demonstrated considerable high expression in some samples. Of the analysed liposarcomas, 17/33 (52%) revealed an increased expression of PTX3 compared to normal tissues underlining the overexpression of PTX3 in liposarcoma tissue ($p = 0.02$). In detailed analysis, high PTX3 expression in immunohistochemistry was unrelated to the FUS/CHOP fusion transcript status of the respective tumour ($p = 0.56$ McNemar's test; Table 3).

3.5. *Western blot confirms enhanced expression of PTX3 in human liposarcoma*

In order to support the data collected in immunohistochemistry, we performed Western blot experiments with the identical set of genes evaluated. In summary, the majority of analysed proteins lacked differential expression. However, we found PTX3 to be differentially expressed in the various tissues, with a distinct overexpression in 16 of the 34 liposarcomas evaluated (47%, $p = 0.01$) (Table 1 and Fig. 3). While we observed an increase of PTX3 expression in WB in liposarcomas in general, no difference was observed between FUS/CHOP positive and FUS/CHOP negative tumour tissues ($p = 0.78$ McNemar's test; Table 3).

Comparing the overexpression of the individual tumour specimen in real time PCR, IHC and Western blot experiments, 21/33 samples demonstrated increased expression within the tumour in at least two of the three analytic processes (Table 1).

4. Discussion

Chromosomal translocations are an attractive aim for tailoring therapeutical interventions in the era of molecular tumour analysis.[13] In the majority of translocations found in solid tumours, a transcript is generated by the fusion of two genes originally localised on different chromosomes. Therefore, the resulting fusion gene is solely expressed in tumour cells making them clearly distinct from normal cells. This distinction has been used to confirm conventional histopathology by fusion transcript detection[14] and to demonstrate minimal residual disease utilising an RT-PCR approach selectively processing fusion transcripts.[8,15] In contrast to some

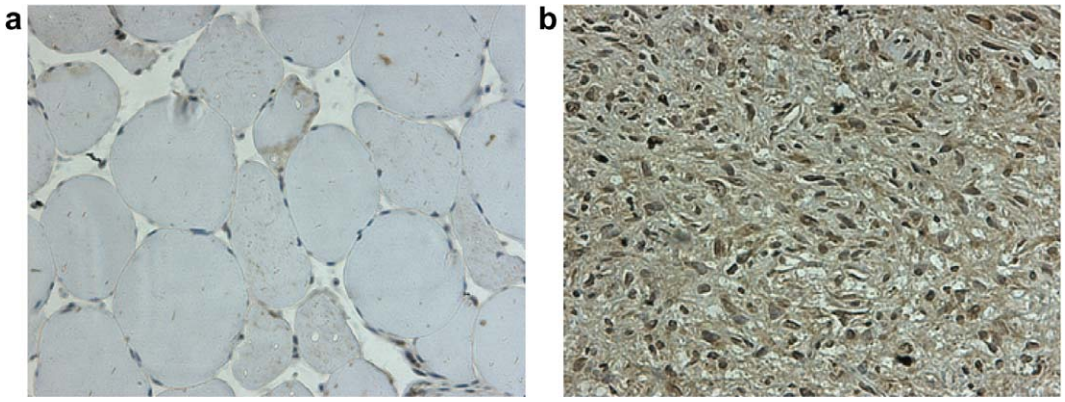

Fig. 2 – Immunohistochemistry of liposarcoma specimen with corresponding normal fat tissue. (a) immunohistochemistry of normal fat tissue stained with PTX3, resection margin of B. Neither cell nucleus nor cytoplasma reveal any staining. (b) immunohistochemistry of a pleomorphic liposarcoma (1a) stained with PTX3. Intense staining of more than 50% of cell nucleus and marked staining of the cytoplasm.

Table 3 – No correlation between PTX3 expression and FUS/CHOP in immunohistochemistry and western blot analysis

FUS/CHOP	Western blot for PTX3		Immunohistochemstry for PTX3	
	positive	negative	positive	negative
Positive	9	6	9	5
Negative	7	12	7	10

Calculation of difference to normal tissue was based on a difference exceeding one category in a scale from 0–4 (see Patients and methods for details).
Abbrev.: FUS/CHOP pos. = liposarcoma with FUS/CHOP fusion transcript; FUS/CHOP neg. = liposarcoma without FUS/CHOP fusion transcript; WB = Western blot; IHC = immunohistochemistry.

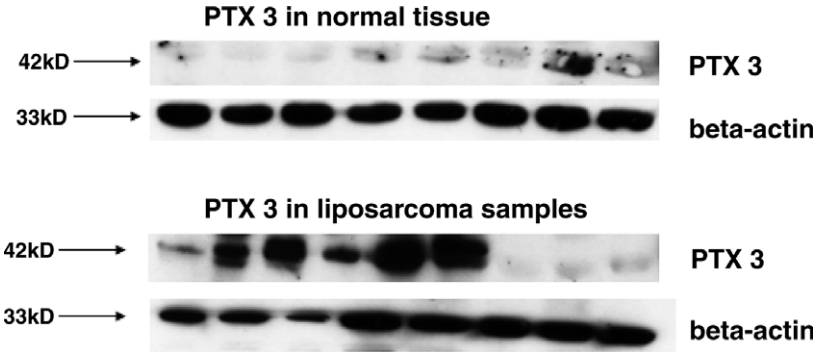

Fig. 3 – Western blot of PTX3 in normal tissue and corresponding liposarcoma.

promising experimental data,[16] therapeutical approaches to influence solid tumour development with interference of fusion transcripts has been disappointing. To focus on how the fusion transcript develops its oncogenic potential, we performed transfection experiments with NIH 3T3 cells and analysed the genes downstream of FUS/CHOP. We found a panel of genes differentially expressed depending on the cotransfection of the FUS/CHOP fusion oncogene. These genes included the pentraxin related gene (PTX3), the osteoblast-specific factor 2 (osf-2), the basic Kruppel-like factor (bklf), the leucoprotease inhibitor, and cyclophilin B.[5]

4.1. *PTX3 is overexpressed in human liposarcoma irrespective of FUS/CHOP*

The vast majority of genes with promising expression profiles in cDNA experiments[5] failed to confirm these early findings in our present immunohistochemistry analysis. No differential expression could be demonstrated in FUS/CHOP positive or FUS/CHOP negative liposarcoma for all but one gene. These data underscore the need for additional analyses to confirm the gene expression data yielded in the experimental transfection setting. The sole gene confirming the data of the cDNA analysis was pentraxin 3 (PTX3 syn.TSG 14), originally described as a cytokine-inducible gene in endothelial cells.[17] The highly significant overexpression of PTX3 was highlighted by the data from real time PCR analysis. Yet, although protein analysis confirmed the high expression of PTX3 in liposarcoma in general, increased RNA levels for PTX3 were not paralleled in all tumours by increased PTX3 protein levels detectable by immunohistochemistry and/or Western blot analysis. This is a well recognised phenomenon and researchers postulate several causes for this observation, such as differing transcription or translation efficacies, stability of the protein product, or insufficient assay sensitivities.[18]

However, the overexpression of PTX3 in liposarcomas was not restricted to FUS/CHOP bearing tumours as suggested by the data from transfection experiments. We found PTX3 to be overexpressed in all subtypes of liposarcoma, although round cell liposarcomas, a histological subgroup of liposarcoma known to carry FUS/CHOP in nearly all cases analysed, showed the highest expression of PTX3. Even more surprising, some non-lipomatous soft tissue sarcomas originally selected as control tumours, also demonstrated high expression of PTX3. It has to be speculated, that PTX3 overexpression could be due to two different pathways, on the one side possibly triggered by FUS/CHOP expression, on the other side by an unknown pathway yet to be determined and obviously not restricted to lipomatous sarcomas. One possible pathway could be an inflammatory response related to the peritumoural tissue, since PTX3 is known to be induceable by inflammation.[19]

4.2. *Is there a rationale for PTX3 involvement in the oncogenesis of liposarcoma?*

While the exact role of the acute phase protein PTX3 is unknown, the group of pentraxins are known to be involved in innate immunity, inflammation, matrix deposition, and female fertility.[19] Further functions of pentraxins concern apoptosis. A subform of PTX3 binds to apoptotic cells and regulates their clearance by antigen-presenting dendritic cells.[20] Aside from its potential role in apoptosis, PTX3 has been found to have a high affinity to the angiogenic fibroblast growth factor-2 (FGF-2). PTX3 was able to selectively inhibit activation of FGF-2 and may therefore influence angiogenesis in the process of malignant transformation.[21]

A striking crosslink to the ubiquitous overexpression observed in liposarcomas is the recent description of PTX3 as a TNF alpha inducible protein in adipocytes. While PTX3 expression in the resting adult adipocyte was negligable, TNF alpha was able to dramatically increase PTX3 expression in the adipose cell. The PTX mRNA fraction was demonstrated in stromal vascular fraction of adipose tissue, but not in the adipocyte itself.[22] It has been speculated that PTX3 has specific features in the commitment of mesoderm precursor cells to define specific lipoblast differentiation. Since liposarcomas are thought to develop from mesenchymal precursor cells, the overexpression of PTX3 both in these stem cells and liposarcomas may well support the theory of liposarcomas being derived from mesenchymal stem cells. Besides this association, one could speculate that interference with a functional NF-κB site in the promoter region of PTX3 might be a missing link in the puzzle.[23] Very recent work was able to demonstrate the interference of FUS/CHOP with interleukin 6 and 8 suggesting the fusion gene might affect NF-κB promoter activation.[24] However, profound knowledge of the possible interaction is yet missing.

4.3. *Are there additional candidate genes as oncogenic effectors of FUS/CHOP?*

Subtractive hybridisation techniques led to the identification of six genes characterised by an overexpression in t (12;16) liposarcomas. The glia-derived nexin gene (PN-1), which is a serine protease inhibitor and neuronatin, involved in brain development, are two of these genes. While PN-1 might parallel the expression of SLPI,[25] overexpression of the latter has been explained by a neural origin of the FUS/CHOP bearing liposarcoma.[26] Additionally, the overexpression of RET was observed, a gene known for inducing variant tumour syndromes if affected by germ line mutations.[26] In contrast to the reported data, we were unable to confirm an epistatic relationship between RET and liposarcomas in immunohistochemistry or Western blot experiments (data not shown). Utilising representational difference analysis, another group defined a close relationship between FUS/CHOP and DOL54/MSF (megakaryocyte stimulatory factor).[27] Due to a lack of available antibodies for most of the described genes, these could not be included in the current analysis.

Although human soft tissue liposarcoma is the most common solid tumour in adults regularly bearing chromosomal translocations, the pathway of fusion transcript generated oncogenesis is poorly understood. Here, PTX3 could be identified as a potential relevant gene in the context of liposarcoma development in general. The clarification of detailed interactions of genes including PTX3 with or without dependence on fusion transcript activation should ultimately lead to novel therapeutic interventions.

Conflict of interest statement

None declared.

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