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Genetic Aberrations in Soft Tissue Leiomyosarcoma

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Abstract

Leiomyosarcoma is a malignant mesenchymal tumor composed of cells showing smooth muscle differentiation. This tumor usually occurs in middle-aged or older adults, and forms a significant percentage of retroperitoneal, vascular, extremity and uterine sarcomas. Leiomyosarcomas are most often associated with complex karyotypes with numerous chromosomal gains and losses. Some of these cytogenetic and molecular genetic aberrations correlate with histopathologic features and clinical outcomes. Identification of genetic alterations with specific identification of oncogenes and tumor suppressor genes may lead to additional insights into the tumorigenesis of leiomyosarcoma and the opportunity to confer the benefits of targeted therapy.

Introduction

Bone and soft-tissue sarcomas are uncommon neoplasms representing no more than 1 % of malignant tumors [1]. Some of these tumors, such as synovial sarcoma, Ewing's sarcoma, and osteosarcoma, occur most often in adolescents or in young adults; other sarcomas, however, such as leiomyosarcoma or well-differentiated liposarcoma, are more frequent in older individuals. On the basis of histology alone, there are more than fifty distinct types of sarcoma [2]. From the molecular point of view, these neoplasms can be bifurcated into two major groups: (a) sarcomas showing specific, recurrent genetic alterations and relatively simple karyotypes (such as the *EWSR1-FLI1* gene fusion in Ewing's sarcoma), and (b) sarcomas showing multiple and often variable gene alterations and very complex karyotypes, such as leiomyosarcoma and osteosarcoma [3].

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Leiomyosarcoma is a malignant tumor composed of cells showing distinct features of the smooth muscle lineage. It usually occurs in middle-aged or older adults. These tumors arise most commonly in five distinct anatomic sites: (1) retroperitoneum; (2) deep extremity; (3) uterus; (4) blood vessels; and (5) superficial dermis. When confined to the dermis, this latter group has an indolent clinical course and rarely metastasizes. Leiomyosarcoma constitutes a significant percentage of retroperitoneal and pelvic sarcomas. It is comparatively less common at other sites, accounting for at most 10-15% of extremity sarcomas. The deep leiomyosarcomas frequently arise in association with the smooth muscle wall of a vessel or a tubular digestive organ. Uterine leiomyosarcomas arise in the context of the myometrium. Deep leiomyosarcoma is a deadly cancer with significant mortality associated with pulmonary metastases. Risk for local recurrence, metastasis and tumor-specific mortality correlates with the three-tiered histologic grade (low, intermediate, high) assigned under the French sarcoma grading system guidelines[4], though these criteria are not clearly applicable to the uterie and dermal categories.

Leiomyosarcoma often present as an enlarging mass. Imaging studies demonstrate a nonspecific soft tissue mass but are helpful in delineating the relationship to adjacent structures, particularly in the retroperitoneum. The typical histological pattern of leiomyosarcoma is of intersecting, sharply marginated fascicles of spindle cells with abundant eosinophilic cytoplasmic and elongated (cigar-shaped) nuclei. The great majority of leiomyosarcomas are reactive for SMA, desmin, and h-caldesmon on immunohistochemistry, though none of these markers are absolutely specific for smooth muscle differentiation.

Until recently years gastrointestinal stromal tumors (GIST) were not clearly delineated from leiomyosarcoma. In contrast to GISTs where >90% of cases are positive for c-kit protein in immunohistochemisty analysis, leiomyosarcoma only rarely expresses c-kit and even then, only at low levels [5,6]. The signature mutation and overexpression of *KIT* and *PDGFRA* genes provide a target for selective therapy with the kinase inhibitor imatinib mesylate (Gleevec) [7]. In contrast, therapeutically relevant targets in leiomyosarcoma have yet to be discerned. Currently, leiomyosarcoma is not amenable or unlikely to be controlled by surgery alone and is treated by conventional cytotoxic chemotherapy at many centers, yet only 50% of patients respond with less than 10% long-term survival [6]. Clearly, efforts are needed to reveal recurrent genetic and molecular changes in leiomyosarcoma that may lead to improved therapeutic interventions.

Cytogenetic aberrations

The existing published data show that the cytogenetic and molecular genetic changes in leiomyosarcoma are complex [8]. Wang et al. [9] in 2001 examined karyotypes of about 100 leiomyosarcomas and found that most karyotypes were complex and there were no consistent, recurrent aberrations demonstrated at the chromosomal level. DNA copy number changes in 29 leiomyosarcomas were further investigated by comparative genomic hybridization (CGH). The most frequent losses were detected in 10q (20 of 29) and 13q (17 of 29), the regions where tumor suppressor genes *PTEN* and *Rb* reside. In contrast to many other tumor types such as colon carcinoma, loss of p53-containing 17p was not frequently seen. Rather, the most frequent gains were detected in 17p (16 of 29). The most frequent high-level amplifications were detected in 17p (7 of 29) and 8q (6 of 29). The extent of cytogenetic changes was associated with tumor size. Small tumors, less than 5cm in diameter, displayed fewer changes per sample (3 to 11; mean, 7) than larger tumors which the diameters are more than 5cm but less than 20cm (4 to 22; mean, 13). The number of gains further increased from small tumors to very large tumors (>20cm). Interestingly, gains in 16p were detected in all small tumors but were infrequent in large and very large tumors (27% and 11%, respectively). Similarly, gains and high-level amplifications in 17p were more common in small (80%) than in very large tumors

(33%), suggesting that the small and large tumors could represent distinct types of leiomyosarcoma and the large tumors do not necessarily progress from the small tumor type or at least do not derive from the same clones. Gains in 1q, 5p, 6q, and 8q were detected in large and very large tumors, and gains in 6q and 8q were more likely to occur specifically in very large tumors (8/9, 89%) making these two regions candidates for activating oncogenes [10]. The oncogenes, MYC, located on 8q24, and MYB, located on 6q22, are likely candidate oncogenes involved. In another study, trisomy 8 was seen in leiomyosarcoma by using a combination of interphase fluorescence in situ hybridization (FISH), dual-color FISH, spectral karyotyping (SKY), and oligonucleotide array CGH, possibly as a mechanism for activation of MYC oncogene [11]. Trisomy eight is seem in other mesenchymal tumors as well.

In the study using the method of CGH by Otaño-Joos et al. [12], 14 cases of leiomyosarcoma were screened for changes in relative chromosome copy number. The most frequent gains were found in 5p15, 8q24, 15q25-26, 17p, and Xp, whereas the most frequent losses were found in 10q and 13q, which respectively harbor tumor-assciated gene such as PTEN and RB [13,14], similar to the Wang report [9]. The sequences on chromosome arm 17p were found to be highly amplified (3/3), with a minimal overlapping region on sub-bands 17p12-p11 by the methods of CGH and Sequencing [12]. Gain of 17p11-12 was also reported in high-grade osteosarcoma as well as in gliomas [15]. The putative oncogenes on 17p are not well characterized. However, COPS3 located in the 17p11-12 region was shown to target p53 protein for proteosome-mediated degradation in osteosarcoma [16]. The investigator reported that in the osteosarcoma where COPS3 was amplified, none of them had MDM2 amplification or p53 mutation, suggesting that COPS3 is an alternative mechanism for p53 inactivation in these tumors. Interestingly, MDM2, which is located on 12q15, was not amplified nor was p53 gene containing 17p deletion in leiomyosarcoma. Therefore, leiomyosarcoma and ostersarcoma may share a common mechanism for inactivation of p53 pathway.

Ten different leiomyosarcomas affecting a single patient over a period of 3 years were studied by Riva et al. [17] to detect nonrandom chromosomal changes with a pathogenetic significance. All tumors were classified as small, including eight that developed before chemotherapy. The diagnoses were based on standard immunohistochemical methods for smooth muscle tumors. Scoring of 613 metaphases revealed monosomy of chromosome 22 in six of the leiomyosarcomas, monosomy of chromosome 19 in three, and deletion of chromosome 19p in all ten. The frequent deletion of chromosome 22 reported in this study was not found in the larger studies discussed in the above paragraph. An interesting note is that monosomy of chromosome 22q has been reported as a frequent event in GIST [18]. Therefore, it is possible some of these tumors classified as leiomyosarcoma in that study may actually be GIST if they were re-evaluated. The recurrence of the 19p deletion, where p16INK4a and ARF located, in a subset of tumor cells from the analyzed leiomyosarcomas as well as other high-grade sarcomas suggests that this structural aberration is a significant change in the development of these tumors [19]. Because p16INK4A and ARF are critical regulators of RB and p53 tumor suppressor gene functions, 19p deletion represents anther major mechanism for inactivation of two important tumor suppressor genes.

In anther study, 27 leiomyosarcomas were characterized by CGH [20]. Significant losses of chromosome 13 were detected in 19 (70.4%) of the 27 tumors, with a putative common region of loss in bands 13q14-21, where the *RB* tumor suppressor gene is located. Thus, in leiomyosarcoma, there are two major mechanisms for RB inactivation, deletion of *p14INK4a* and *RB* gene itself. Consistently, the Rb-cyclin D pathway was analyzed by studying the Rb gene and protein, p16MTS1/INK4A gene and protein, cyclin D1Prad1/bcl1 and cyclin D3 proteins. Abnormalities involving this pathway were found in 16 of the 23 leiomyosarcomas [21].

In an earlier report by Mandahl et al. [22] in 2000, the investigators searched for recurrent chromosome aberrations in 45 leiomyosarcomas and attempted to correlate these with morphological and clinical parameters. The breakpoints were widely scattered, and none of the recurrent breakpoints or losses displayed any predilection for any of the morphologic subtypes. When numerical and unbalanced structural changes were combined, the most frequently lost segments were 3p21-p23 (11 cases), 8p21-pter, 13q12-q13, 13q32-qter, 1q42-qter, 2p15-pter, 18p11, 1p36, 11q23-qter, and 10q23-qter. The most frequent gain was 1q12-q31. There was a greater frequency of losses in 1p and 8p and a lower frequency of losses in 10q and 13q in tumors that had metastasized than in localized tumors. The profiles of the chromosomal changes in this early study were quite different from later reports. One possible explanation is that some of the 45 leiomyosarcomas studied in that series would have been identified as GIST upon re-evaluation. Thus, caution is needed when evaluating past reports on leiomyosarcomas.

Little is known about how the cytogenetic changes are associated with clinical outcome in leiomyosarcoma and most studies to attempt this association suffer from small sample sizes. Peng et al. [23] found that loss of heterozygosity (LOH) occurred more frequently at D3s1295 and D3s1289 on chromosome 3. The *FHIT* tumor suppressor gene in this region may be a critically affected gene; significant difference found in LOH incidence among different grades, sizes, and locations of leiomyosarcomas.

Losses in 1p36 and 8p21-pter may be associated with increased risk of metastasis. Wang et al. [24] studied 28 leiomyosarcoma samples of similar grade using CGH and DNA flow cytometry and identified a difference in survival time associated with ploidy status and the number of chromosomal aberrations. The average survival time was shown to decrease with an increase in chromosomal aberrations as is commonly observed in most sarcomas. Similar correlation between the extent of chromosomal aberrations with survival has also been seen in other types of cancers such as squamous cell carcinoma and colon cancer [25,26]. This is consistent with the hypothesis that cancer progression is a process of accumulated genetic alteration. The average survival time was shorter in the near-tetraploid group than in the diploid and triploid group. Gain of 5p14-pter was significantly more common in near-tetraploid tumors. The survival time of patients with near-tetraploidy together with gain of 5p14-pter was reduced, and 50% died within the first year. Furthermore, loss of 13q14-q21, where tumor suppressor gene *RB* resides, was significantly more frequent in patients surviving less than 5 years than in those surviving more than 5 years. These results suggest that 13q14-q21 loss and 5p14-pter gain at diagnosis of leiomyosarcoma could be used to identify patients who are likely to have a shorter survival time and who might benefit from early treatment intensification.

Hu et al. [27] analyzed ten primary, five metastatic, and two recurrent extrauterine leiomyosarcomas. Genomic imbalances were detected in 15 of the 17 tumors examined. Large tumors and tumors with metastasis showed 10q deletions, and a gain of 5p was detected only in histologically high grade tumors. These findings suggest that loss of 10q (which harbors the *PTEN* gene) and gain of 5p (which harbors the *cyclinA* gene) are associated with aggressive behavior in leiomyosarcomas.

The cytogenetics data that have been published so far are complex, limited, and incomplete, Larramendy et al. [28] performed array CGH analysis with a 13K cDNA clone array on a pool of 14 leiomyosarcoma cases to obtain gene-level information about the amplified and deleted regions that may play a role in development and progression of these tumors. The CGH results indicated that 2,218 genes were involved in 25 altered chromosomal regions; nine regions in low-grade leiomyosarcomas, 12 regions in high-grade leiomyosarcomas. Minimal common regions of gain (15q26 approximately qter and 17p13.1 approximately q11) and loss (6p12 approximately p21.3 and 13q14.3 approximately qter) were shared by low- and high-grade

leiomyosarcomas. The frequency of DNA copy number gains in high-grade leiomyosarcomas was threefold compared to that in low-grade leiomyosarcomas, whereas losses in low-grade leiomyosarcomas were almost twice as frequent as those in high-grade leiomyosarcomas. This pattern, although based on small number of cases, if holds true in larger validation studies, would suggest that loss of tumor suppressor genes is the initiating event for leiomyosarcoma development and activation of oncogenes is critical for tumor progression.

In summary, as shown in Table 1, there are frequent numerical changes in leiomyosarcoma, including gain of material from chromosomes 1q12-q31, 1q21, 1p3, 5p15, 6q, 8q24, 15q12-15, 15q25-q26, 16p, 17p, 17q, 19, 20q, 22q, and Xp, and losses from 1q42-qter, 1p36, 2p, 2q, 2p15-pter, 3p21-p23, 4q, 8p21-pter, 9p, 10p, 10q23-qter, 11p, 11q23-qter, 13q12-q13, 13q14-21, 13q32-qter, 16q, and 18p11, and identified regions of amplification, e.g., 1q21, 5p14-pter, 8q, 12q13-15, 13q31, 17p11, 19p13, and 20q13. Trisomy 8 and LOH on chromosome 3p14.2-23 have also been reported. Relationships between tumor size and chromosomal changes have been observed, such as gains of 16p and 17p in smaller tumors and gains of 6q and 8q in larger tumors. Loss of function of tumor suppressor genes or activation of oncogenes (or both) resulting from these copy number changes might play important roles in development of soft tissue leiomyosarcomas. With the advancement of high resolution microarray based array CGH technology, future studies with larger cohort of samples will further refine the exact events at chromosomal level in leiomyosarcomas.

Molecular aberrations

Little is known about the underlying molecular determinants driving soft tissue leiomyosarcoma inception, proliferation, and metastasis. Although 17p which harbors *p53* tumor suppressor gene has been found to be amplified rather than deleted in leiomyosarcoma [9], frequent deletion of 19p, where *ARF* is located, suggests inactivation of *p53* tumor suppressor gene in leiomyosarcoma via a different mechanism. Amplification of the *COPS3* gene located in the 17p11-12 region was proposed to target p53 protein for proteosome-mediated degradation in osteosarcoma in a manner similar to mdm2 [16]. Nevertheless, mutation of p53 gene itself has also been found in leiomyosarcoma. Pollock et al. [29] transduced wild-type (wt) *p53* into human-derived leiomyosarcoma cell line SKLMS-1 (which bears a mutated *p53* gene) to investigate whether this single change could alter the malignant phenotype. SKLMS-1 cells stably expressing wt *p53* had decreased cell proliferation *in vitro*, decreased *in vitro* colony formation in soft agar, and decreased tumorigenicity in mice *in vivo*. Flow cytometric analysis of cell cycle components demonstrated markedly increased G_1 cell cycle arrest and decreased entry into S phase, which corresponded to induction of p21cip1 protein in the transfected cells.

The expression levels of *p16INK4a* were examined in leiomyosarcoma. In one study, expression of the p16 protein was decreased in 25 of 77 leiomyosarcomas examined [30]. Decreased expression of p16 correlated significantly with large tumor size and was the only independent prognostic factor for unfavorable outcome. To elucidate the mechanisms of inactivation of the *p16INK4a* gene, 49 leiomyosarcomas were examined and no mutation was recognized in any of the cases studied. Eight of 15 cases with decreased expression of p16 protein revealed methylation of the *p16INK4a* gene promoter. Promoter hypermethylation correlated closely with decreased expression and poor prognosis. These results suggest that decreased expression of p16 protein can be considered an independent, reliable prognostic parameter in patients with leiomyosarcoma. *p16INK4a* gene promoter methylation was shown to have a strong association with inactivation of the gene.

Death-associated protein (DAP) kinase is a 16-kDa calmodulin-dependent serine/threonine kinase that carries a death domain at its *C*-terminus. DAP kinase functions as a positive

mediator of apoptosis that is induced by interferon-gamma, Fas, tumor necrosis factor, or deregulated oncogenes such as c-*Myc* or *E2F-1*. Recent studies [31] suggest that DAP kinase is involved in tumor metastasis and that it can be inactivated by methylation of CpG islands in the promoter region of the gene in some human tumors. To investigate the potential role and the alteration of the DAP kinase gene in leiomyosarcoma, Kawaguchi et al. [32] first searched for homozygous deletion and promoter hypermethylation of the DAP kinase gene in 45 leiomyosarcomas. Promoter methylation was recognized in ten of 45 cases, and homozygous deletion of 9q34, where DAP kinase gene is located, was detected in three of 45 cases. Decreased expression of DAP kinase protein was recognized in 13 of 45 cases. Seven of these 13 cases revealed promoter methylation or homozygous deletion of DAP kinase, and the methylation status or homozygous deletion of its gene showed a close correlation with decreased DAP kinase expression. Mutation of *p53* was detected in 11 of these 45 leiomyosarcoma cases. Cases with DAP kinase alteration or *p53* mutation showed a close correlation with high grade (French Federation of Cancer Centers) and/or poor prognosis. Therefore, promoter methylation of DAP kinase may present an important mechanism for decreased DAP kinase expression and reduced apoptosis in leiomyosarcoma [31].

Overexpression of the transcription factor E2F-1 has been shown to induce apoptosis in a variety of carcinoma cells and also is known to inactivate murine double minute protein 2 (MDM2), a factor associated with poor prognosis in soft tissue sarcomas. Vorburger et al. [33] utilized a replication-deficient adenovirus carrying the *E2F-1* gene (Ad5E2F) to induce E2F-1 overexpression in the *p53*-mutated leiomyosarcoma cell line SKLMS-1. E2F-1 overexpression within 48 hours following infection with Ad5E2F led to cell apoptosis. *In vivo* treatment of SKLMS-1 tumor-bearing BALB/c mice with intratumoral injections of Ad5E2F viral particles resulted in significant inhibition of tumor growth compared with treated control animals. Complete disappearance of all tumors was seen in two of seven mice in the Ad5E2F-treated animals. Immunohistochemical analysis of tumor specimens showed overexpression of E2F-1 in Ad5E2F-treated tumors, with upregulation of the double-stranded RNA activated protein kinase PKR. These investigations demonstrated that adenovirus-mediated overexpression of E2F-1 results in upregulation of PKR and significant growth suppression of leiomyosarcomas *in vivo*.

Gene expression in leiomyosarcoma was examined [34] using microarray analysis arrays containing approximately 12,000 known genes and 48,000 expressed sequence tags. Six genes, including cyclin-dependent kinase (CDK) inhibitor 2A, diaphanous 3, doublecortin, calpain 6, interleukin-17B, and proteolipid 1, were found to be overexpressed in leiomyosarcoma compared with normal myometrium and 18 other tissues. Several genes were found to be underexpressed in leiomyosarcoma, including alcohol dehydrogenase 1A-polypeptide, alcohol dehydrogenase 1B- polypeptide, insulin-like growth factor 1, c-*jun*, c-*fos*, and *TU3A*. These changes in gene expression may yield clues to the pathophysiology of leiomyosarcoma. Ragazzini et al. [35] evaluated amplification and overrepresentation of the *CDK4*, *MDM2*, *GLI*, and *SAS* genes of the 12q13-15 region, in a group of soft tissue sarcomas including leiomyosarcomas using quantitative real-time PCR and immunohistochemical staining, to identify genomic alterations. One or more of the 12q13-15 genes was altered in 13 of the 29 leiomyosarcomas evaluated (45%). Among the leiomyosarcomas, most cases with *CDK4*, *MDM2* or *GLI* gene alteration also showed a simultaneous high level of expression of the protein product. These results indicate that amplification or overrepresentation of genes at the 12q13-15 region is often involved in leiomyosarcoma. However in a separate study [36] with the methods of real-time PCR and FISH by Shimada et al., none of the leiomyosarcomas had the *MDM2* and *CDK4* amplifications. So the expression and significance of *MDM2* and *CDK4* in leiomyosarcoma requires further investigation.

Aberrant methylation is one of the main mechanisms of tumor suppressor gene inactivation and oncogene activation in carcinogenesis. In the study by Seidel et al. [37], the methylation status of *RASSF1A*, *p16*, *MLH1*, *MSH2*, and *ERalpha* was investigated in 18 leiomyosarcomas and several other sarcomas. *RASSF1A* methylation was more frequent in leiomyosarcomas than in malignant fibrous histiocytomas and liposarcomas. In 7 of 81 (9%) STSs, inactivation of *MLH1* was detected. Hypermethylation of *MLH1* was found in 3 of 21 (14%) liposarcomas, in 1 of 18 (6%) leiomyosarcomas and in 1 of 17 (6%) MFHs. No *MSH2* hypermethylation was detected. In a univariate Cox proportional-hazards regression model, the risk of a tumor-related death was significantly increased for patients with soft tissue sarcoma with methylated *RASSF1A*. The data indicate that inactivation of *RASSF1A* is a common event in soft tissue sarcoma, especially in leiomyosarcoma. Promoter methylation and homozygous deletion of the *PTEN* gene were also found in cases of leiomyosarcoma, as was mutation of the gene. Although a further detailed analysis of a larger number of cases is still necessary, these results suggest that PTEN expression may be a regulator of cell proliferation in patients with leiomyosarcoma [38].

As shown in Table 2, analysis of the genes and proteins in the Rb-cyclinD pathway (*RB1*, *CDKN2A*, *CCND1*, and *CCND3*) revealed that these frequent abnormalities may correlate with poorer prognosis in leiomyosarcomas. CDK inhibitor 2A, diaphanous 3, doublecortin, calpain 6, interleukin-17B, and proteolipid 1 were found to be overexpressed in leiomyosarcoma. From a therapeutic perspective, overexpression of *E2F-1* results in upregulation of PKR, induction of apoptosis, and significant suppression of growth of leiomyosarcomas *in vivo*. Promoter methylation and homozygous deletion of the *PTEN* and DAP kinase genes, and methylation of *RASSF1A* and the *p16INK4a* gene promoter, appear to be the most important molecular genetic alterations. Amplification at a number of loci suggests candidate genes in these regions, including *MYC*, *MYB*, *COPS3*, *MDM2*, *GLI*, *CDK4*, and *SAS* at 12q13-15 and *FLF* and *PRUNE* at 1q21 [8,39].

Organ specific gene activities

There appear to be organ-related phenotypes among leiomyosarcomas. The uterine leiomyosarcoma have different clinical and genetic features from the other leiomyosarcomas. Cho et al. showed that uterine leiomyosarcoma had specific gains and losses by the methods of genome-wide array-based comparative genomic hybridization (array-CGH) and fluorescence in situ hybridization (FISH). The regions of high level gain were 7q36.3, 7q33-q35, 12q13-q15, and 12q23.3, while the regions of homozygous loss were 1p21.1, 2p22.2, 6p11.2, 9p21.1, 9p22.1, 14q32.33, and 14q32.33 qter. The regions with high level gains included HMGIC, SAS, MDM2, TIM1 genes [40]. Wang et al. found that KIT is expressed in uterine leiomyosarcoma, thus adjunctive diagnostic studies using c-kit may be useful in distinguishing leiomyosarcomas from benign leiomyomas in uterine tumors that offer uncharacteristic features [41]. Uterine leiomyomas are not associated with activating mutations in *KIT*. In a small portion of uterine leiomyosarcomas associated with the hereditary leiomyomatosis and renal cell cancer (HLRCC), germline mutations and biallelic inactivation in fumarate hydratase (FH) gene at 1q43 was proposed to play a role in the pathogenesis of sporadic early onset ULMSs [42-43]. These types of leiomyosarcoma have different patterns of tumorigenesis, progression, and different prognosis, thus may require different methods of clinical management. Future studies will provide a clearer picture regarding the site-specific genetic and molecular events in leiomyosarcoma.

Gene expression profiling studies

The focus of this review is on genetic changes in leiomyosarcoma at gene copy number changes and related molecular alterations. However, it should be noted that gene expression profiling

studies using expression microarrays have also been studied by several groups. Nielsen et al [44] showed that gene expression profiles can be used to classify different types of soft-tissue tumors and improve on the histology-based classification method. Pathway analysis of the gene expression profiles of sarcomas could also provide insight into sarcoma biology and give us clues about cellular differentiation and oncogenic pathways active in these tumors as well as potential biomarkers and therapeutic targets [45]. The application of a novel classification algorithm to a large set of gene expression profiles from GIST and leiomyosarcoma samples led to a discovery of a gene pair classifier that highly accurately differentiate between these two types of sarcomas [7]. Integration of gene expression profiles and gene copy number profiles are underway and will lightly provide additional information regarding the molecular basis of leiomyosarcoma development.

Summary

Soft tissue leiomyosarcomas show multiple gene alterations and very complex karyotypes, including numerous gains and losses. Some of the cytogenetic and molecular genetic aberrations are correlated with the clinical pathologic features and with prognosis in small pilot studies with limited clinical information. Further exploration of aberrations of the oncogenes and the tumor suppressor genes in soft tissue leiomyosarcomas with a large cohort of patient samples and with high resolution genomic technologies coupled with molecular and functional validation promises to lead to further insights into the basis of tumorigenesis may perhaps open the door for the application of targeted therapeutics approaches for these neoplasms.

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Table 1
Cytogenetic aberrations and correlations with clinical or/and pathological features

Cytogenetic changes	Chromosomes affected	Specific sites	Special sites	Clinical or/and pathologic features
Chromosomal imbalance aberrations: **gain**	1, 5, 6, 8, 15, 16, 17, 19, 20, 22, X	1q12-q31, 1q21, 1p3, 5p14-pter, 5p15, 6q, 8q24, 15q12-15, 15q25-->q26, 16p, 17p, 17q, 19, 20q, 22q, Xp	5p14-pter 1q, 5p, 6q, 8q 5p 16p, 17p	Reduced survival time More frequent in large and very large tumors Aggressive behavior More frequent in small tumors
Chromosomal imbalance aberrations: **loss**	1, 2, 3, 4, 6, 8, 9, 10, 11, 13, 16, 18, 19	1q42-qter, 1p36, 2p, 2q, 2p15-pter, 3p21-p23, 4q, 6q, 8q, 8p21-pter, 9p, 10p, 10q, 10q23-qter, 11p, 11q23-qter, 13q12-q13, 13q14-21, 13q32-qter, 16q, 18p11, 19p13	1p36, 8p21-pter 13q14-21 10q 19p13	High risk of metastasis Shorter survival time Aggressive behavior Development of leiomyosarcoma
Chromosomal imbalance aberrations: **amplification**	1, 5, 8, 12, 13, 17, 19, 20	1q21, 5p14-pter, 8q, 12q13-15, 13q31, 17p11, 19p13, 20q13	17p	More frequent in small tumors
LOH of D3s1295 and D3s1289	3	3p14.2-23	3p14.2-23	No correlation with grade, size, location May harbor tumor suppressor gene
Chromosomal imbalance aberrations	8	Trisomy 8	Not clear	Not clear
Total	**25 chromosomes**	**2,218 genes**	**2,218 genes**	**DNA copy number gains more frequent in high-grade leiomyosarcomas** **DNA copy number losses more frequent in low-grade leiomyosarcomas**

LOH, loss of heterozygosity

Table 2
Molecular aberrations and correlations with clinical or/and pathological features

Involved genes	Gene aberrations	Protein expression	Genetic alterations	Clinical or/and pathological features
				Decreased cell proliferation *in vitro*
			Transfect *wt p53* into SKLMS-1 cells (bearing mutated *p53* gene)	Decreased colony formation in soft agar *in vitro*
				Decreased tumorigenicity in mice *in vivo*
				Correlation with high grade (French Federation of Cancer Centers)
p53	Gene mutation	Decreased protein expression	Mutations	Correlation with poor prognosis
				Association with large tumor size
p16	*p16* inactivation	Decreased protein expression	*p16* gene promoter methylation	Independent prognostic factor for unfavorable outcome
			Promoter methylation	Correlation with high grade (French Federation of Cancer Centers) and poor prognosis
	DAP kinase inactivation	Decreased protein expression	Homozygous deletion	
DAP kinase			Transfect *E2F-1* into SKLMS-1 cells (bearing mutated *p53* gene)	Bcl-2 high expression, E2F-1 high expression
				Tumor apoptosis
			Treatment of SKLMS-1 tumor-bearing BALB/c mice with intratumoral injections of Ad5E2F	PKR expression upregulation
E2F-1				Tumor growth inhibition
RASSF1A	Inactivation of *RASSF1A*	Decreased protein expression	Methylation	Increased risk of tumor-related death
			Promoter methylation	Useful indicator of cell proliferation
			Homozygous deletion	
PTEN	Inactivation of *PTEN*	Decreased protein expression	Gene mutation	
CDK inhibitor 2A	Overexpression	Overexpression	Unknown	Unknown
diaphanous 3	Overexpression	Overexpression	Unknown	Unknown
doublecortin, calpain 6	Overexpression	Overexpression	Unknown	Unknown
interleukin-17B, calpain 6	Overexpression	Overexpression	Unknown	Unknown
proteolipid 1	Overexpression	Overexpression	Unknown	Unknown
alcohol dehydrogenase 1A and 1B polypeptide	Underexpression	Underexpression	Unknown	Unknown
IGF-1, c-jun, c-fos, TU3A	Underexpression	Underexpression	Unknown	Unknown
MDM2, CDK4	Overexpression	High protein expression	Amplification	Unknown