

Smooth Muscle Tumors - Molecular Biology

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Uterine Leiomyoma

The majority of uterine leiomyomas (ULM) occur sporadically, however there appears to be a component of genetic susceptibility to the development of ULM in some cases, as familial aggregation has been noted [1]. The syndrome of multiple cutaneous and uterine leiomyomatosis/hereditary leiomyomatosis and renal cell carcinoma is associated with the development of ULM [2-4]. Patients with this syndrome have germline inactivating mutations in a single copy of the fumarate hydratase (FH) gene and are susceptible to the development multiple cutaneous leiomyomas (at young age) and symptomatic ULM [5], in addition to renal cell carcinomas [6]. Loss of FH through a somatic mutation has also been reported in occasional non-syndromic (sporadic) cases of ULM [7].

By cytogenetic and conventional FISH analysis, most ULM display normal karyotypes [8]. Approximately 40% of ULM have non-random cytogenetic changes but in contrast to LMS, these alterations typically involve only a small number of chromosomal regions and are less complex in nature [8,9]. The most frequent cytogenetic alterations are t(12;14), deletion of 7q and chromosome 12 trisomy, detected in approximately 20%, 17% and 10% of cases respectively [4,8,10]. Other less frequent cytogenetic alterations include 6p and 10q rearrangement and deletion of 3q. Though not well substantiated, there appears to be a tendency for the karyotypically abnormal tumors to be more cellular and/or mitotically active histologically, and to be more frequently intramural or subserosal in location [11,12]. t(12;14) translocation is specific for leiomyoma and involves HMGA2, a putative DNA binding transcriptional regulator. This gene is frequently rearranged in other mesenchymal tumors such as aggressive angiomyxomas and lipomas [13-15]. Other types of structural rearrangements involving HMGA2 have also been described for ULM [8]. Further analysis has revealed that the full coding sequence of HMGA2 is typically retained regardless of the type of rearrangement and it appears that these rearrangements result in increased levels of functional active HMGA2. Furthermore, increased expression of HMGA2 at both mRNA and protein levels is commonly observed in ULM, with the highest levels in tumors with t(12;14)[16]. HMGA2 therefore appears to play a central role in the development and progression of at least a subset of ULM. Diagnostically, DNA copy number gain and increased expression of HMGA2 gene are also observed in uterine leiomyosarcomas (LMS) [17,18], and cannot be used reliably to predict benignancy or malignancy in uterine smooth muscle tumors. Even though t(12;14) has not been reported to occur in uterine LMS to date, it lacks sensitivity for detection of ULM.

In keeping with the cytogenetic observations, conventional or array comparative genomic hybridization (CGH) analyses shows no alterations in gene copy number, or only a few foci of genomic gains or losses, in contrast to the multiple complex genomic aberrations observed for uterine LMS [17,19,20]. Overall, no aberrations common to both ULM and uterine LMS were found.

The gene expression profile of ULM has also been examined in detail, particularly in comparison to that of normal myometrium, and many differentially regulated genes implicated in increased cell growth/proliferation and increased extracellular matrix deposition have been identified by these analyses [1,9,21-24]. Genes implicated in growth stimulation include upregulation of IGF2 and PKCB1 and genes implicated in increased ECM deposition include upregulation of TGFB3 and MMP11. ESR1 is also frequently upregulated in ULM and this is in keeping with the consistent immunohistochemical expression of estrogen receptor as well as progesterone receptor in ULM. It is now believed that both estrogen and progesterone stimulation are important in promoting the growth of ULM [25-27]. Another emerging type of high throughput genetic analysis involves profiling of microRNA (miRNA) expression patterns in tumor samples. MiRNAs are 20~25 nucleotide non-coding RNAs that inhibit the translation of targeted mRNAs, and represent an important epigenetic regulatory mechanism. A few studies have examined the miRNA expression profiles of ULM and found dysregulation of several miRNA in comparison to matched myometrium samples. Most notably, downregulation of Let-7 was observed in ULM, particularly in larger size tumors [28,29]. Let-7 is able to suppress HMGA2 expression [30] and the downregulation of Let-7 seen in large ULM may represent another important mechanism underlying increased HMGA2 expression. Several studies have also compared the mRNA and miRNA expression profiles between ULM and LMS. ULM and uterine LMS generally exhibit gene and miRNA expression profiles that are significantly different from each other. The details of these comparisons will be addressed below, in the LMS section.

Smooth Muscle Tumor of Uncertain Malignant Potential (STUMP)

Little is known regarding the genetics of STUMP. One study reported similar degrees of allelic loss involving common tumor suppressor genes between STUMP and ULM, both of which were significantly lower than that of LMS [31]. Several studies have characterized STUMPs immunohistochemically in relation to ULM and LMS. The majority of STUMPs demonstrate a Mib-1, p16, ER, PR, p53 immunoprofile that is significantly different from LMS but not from that of ULM [32-37], though the Mib-1 proliferation index is typically slightly greater in STUMP than ULM. However, the great majority of STUMPs studied either lacked clinical follow-up data or showed no evidence of disease recurrence. A recent study found the presence of diffuse p16 positivity in two STUMP cases that later developed metastatic disease. Both cases showed the presence of tumor necrosis but no significant atypia or increased mitotic activity [32]. Clearly, more outcome-associated studies are needed to further examine the utility of these and other molecular markers in predicting the clinical behavior of STUMPs. At present those cases diagnosed as STUMP cannot be distinguished, based on molecular markers, from ULM, and within the groups of STUMPs, there are no well-validated molecular markers of increased risk of recurrence.

Uterine Leiomyosarcoma

The vast majority of uterine LMS are sporadic. Patients with germline mutations in FH (described previously) are believed to be at increased risk for developing uterine LMS, as well as ULM [38,39].

Cytogenetically, uterine LMS are karyotypically complex with structural changes involving a large number of chromosomes [9]. The degree and extent of karyotypic complexity is similar to that of soft tissue LMS [9]. Both conventional and array based CGH analysis have identified chromosomal imbalances in nearly all uterine LMS examined [17,19,20,40,41]. The imbalances typically involve several chromosomes with comparable number of gains and losses present. No consistent patterns of gains or losses have been observed, particular between different studies. In comparison to ULM, LMS showed greater number and complexity of chromosomal aberrations overall, such that uterine smooth muscle tumors with complex cytogenetic or CGH abnormalities are very likely malignant, however, no single change is sufficiently sensitive or specific to be diagnostically useful.

Gene expression profiling analysis has identified several differentially expressed genes in uterine LMS when compared to normal myometrium and/or ULM [21,42,43]. In comparison to normal myometrium or to ULM, uterine LMS shows upregulation of several cell proliferation associated genes including TOP2A, PTTG1, CDKIN2A, UBE2C, MCM2 and FOXM1, indicating presence of greater degree of disruption in cell proliferation/cell cycle control in LMS. Other differentially expressed genes include the upregulation of SPP1, NNMT, CHI3L1, GRN, IL17B and downregulation of ADH1A, IGF1 and CALD1. CDKIN2A encodes the protein p16 and in keeping with the gene expression findings, expression of p16 (nuclear and/or cytoplasmic) is greater in LMS versus ULM [32,34,37]. p16 therefore may prove to be a useful adjunct immunomarker in distinction between malignant and benign uterine smooth muscle tumors. While the data is current limited, the miRNA expression profiles of uterine LMS appears to differ from that of normal myometrium and/or ULM [44,45]. More specifically, miR-221 was found to be expressed at higher levels in LMS compared to ULM and benign metastasizing leiomyomas [44]. Thus, the evaluation for miR-221 level or levels of other dysregulated miRNA by RNA in situ hybridization (ISH) method may prove to be useful in identifying LMS in diagnostically challenging cases.

By both gene expression and immunohistochemical analysis, ER expression has been reported to be present in between a quarter to two-third of uterine LMS [46,47]. Some studies have reported a correlation between immunohistochemical ER and/or PR expression and improved clinical outcome in patients with uterine LMS by univariate analysis [46,48]. Other genes of interest that have been examined in uterine LMS include tumor suppressor genes such as p53 and RB1, and oncogenes such as c-KIT. Abnormalities in p53 in the form of missense mutation and/or loss of heterozygosity (LOH) are common in uterine LMS [49,50]. Detection of p53 abnormalities by immunohistochemistry has also been employed to differentiate between benign and malignant uterine smooth muscle tumors. It has limited utility as only a subset (~50%) of uterine LMS shows significant p53 immunoreactivity. Prognostically, p53 over-expression has been reported to be associated with poor outcome [51,52]. LOH of RB1 is also commonly seen in uterine LMS [50]. Regarding c-KIT, while variable proportion of uterine LMS has been reported to demonstrate c-KIT immunopositivity, no mutations in c-KIT have been identified in uterine LMS [53-55].

While a large number of genetic abnormalities have been identified, the oncogenic mechanisms underlying development of uterine LMS remain elusive. Overall, uterine LMS is a genetically

unstable tumor that demonstrates complex structural chromosomal abnormalities and highly disturbed gene regulation, and this likely reflect the end-state of the accumulation of multiple genetic defects in the process of tumor development. Extrapolating from the experiences in soft tissue LMS, it is unlikely that recurrent disease-driven genetic aberrations (i.e. gene mutation or translocation events) will be uncovered. In comparison to other more common uterine malignancies, uterine LMS bear some resemblance to type 2 endometrial carcinomas and high-grade serous carcinomas of ovary/fallopian tube origin, based on their genetic instability, frequent p53 abnormalities, aggressive behavior, and resistance to chemotherapy. As such, therapies that exploit the underlying genetic instability of uterine LMS may prove to be an effective therapeutic strategy.

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