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Molecular and Clinicopathologic Characterization of Intravenous Leiomyomatosis

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Abstract

Intravenous leiomyomatosis (IVL) is an unusual uterine smooth muscle proliferation that can be associated with aggressive clinical behavior despite a histologically benign appearance. It has some overlapping molecular characteristics with both uterine leiomyoma and leiomyosarcoma based on limited genetic data. In this study, we assessed the clinical and morphological characteristics of 28 IVL and their correlation with molecular features and protein expression, using array comparative genomic hybridization (aCGH) and Cyclin D1, p16, phosphorylated-Rb, SMARCB1, SOX10, CAIX, SDHB and FH immunohistochemistry. The most common morphologies were cellular (n=15), usual (n=11) and vascular (n=5; including 3 cellular IVL showing both vascular and cellular features). Among the immunohistochemical findings, the most striking was that all IVL showed differential expression of either p16 or Cyclin D1 in comparison to surrounding non-neoplastic tissue. Cytoplasmic phosphorylated-Rb was present in all but one IVL with hyalinization. SMARCB1, FH and SDHB were retained; S0X10 and CAIX were not expressed. The most common genetic alterations involved 1p (39%), 22q (36%), 2q (29%), 1q (25%), 13q (21%) and 14q (21%). Hierarchical clustering analysis of recurrent aberrations revealed 3 molecular groups: Group 1 (29%) and 2 (18%) with associated del(22q) and group 3 (18%) with del(10q). The remaining IVL had non-specific or no alterations by aCGH. Genomic index scores were calculated for all cases and showed no significant difference between the 14

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IVL associated with aggressive clinical behavior (extrauterine extension or recurrence) and those without (median scores 5.15 vs 3.5). Among the 5 IVL associated with recurrence, 4 had a vascular morphology and 3 had alterations of 8q. Recurrent chromosome alterations detected herein overlap with those observed in the spectrum of uterine smooth muscle tumors and involve genes implicated in mesenchymal tumors at different sites with distinct morphological features.

Keywords

Intravenous leiomyomatosis; IVL; cellular; vascular; hyaline; angioleiomyoma; microarray; aCGH; molecular; genetics; 1p; 1q; 13q; 22q; 10q; 14q; der(14); 8p; 8q; HMGA2; RB; Cyclin D1; p16; phosphorylated Rb; SOX10; SDHB; CAIX

Introduction

Intravenous leiomyomatosis (IVL) is defined as an intravascular proliferation of benign smooth muscle cells in the absence or outside the confines of a uterine leiomyoma (UL)[1]. In most cases, it represents an incidental finding, but it can be grossly identified. IVL may occasionally grow into extrauterine vessels and rarely reach the right heart through the inferior vena cava leading to increased morbidity and mortality. Overall, the prognosis is generally favorable after surgery, even though recurrences have been reported[2–5].

The molecular knowledge on uterine smooth muscle neoplasms, particularly for UL and uterine leiomyosarcoma (ULMS), has significantly advanced in light of high throughput technologies over the last decade. t(12;14) with *HMGA2-RAD51B* fusion and del(7q) are the most common cytogenetic rearrangements in UL[6–9]. Whole-exome sequencing has revealed *MED12* as the most frequently altered gene with mutations seen in approximately 70% of UL[10], while those without *MED12* mutations are reported to have either *HMGA2* rearrangements and/or complex chromosomal rearrangements[11–13]. *MED12* and *HMGA2* alterations together are present in 80–90% of UL[14]. Other recurrent chromosome rearrangements involve 6p21 (*HMGA1*), 1p, 1q (*FH*), 3q and rarely Xq (*COL4A5-COL4A6*)[15–19]. On the other hand, ULMS have much more complex alterations, including 1p, 2p, 6p, 9p, 10q, 13q, 14q, and 22q deletions and 8, 10, 12q, 17, X chromosome gains, and aneuploidy in addition to mutations and deletions in *RB1, TP53* and *PTEN*, suggestive of genomic instability[20,21].

In contrast to UL and ULMS, only three series have been reported to date focusing on the molecular aspects of IVL. In one study, recurrent 22q deletions (66%) and complex copy number alterations were detected using array comparative genomic hybridization (aCGH)[22]. None of these cases showed the most common *MED12* mutation described in UL, highlighting the importance of structural chromosome rearrangements in the development of IVL. A second study described recurrent 12q14.3 rearrangements by FISH along with HMGA2 overexpression (58%, n=12) and loss of chromosome 22 in two out of three IVL analyzed by karyotyping[23]. Finally, a third study using RNA sequencing (n=5) detected *HOXA13* as a distinctly upregulated gene in UL when compared to IVL or myometrium[24]. These studies suggest that IVL has unique molecular characteristics that partially overlap with both UL and ULMS akin to their intermediate clinical behavior. Herein, we study

genome-wide copy number alterations as well as potential correlation with clinical, histological and immunohistochemical features in a large series of 28 IVL.

Materials and Methods

Following institutional IRB approval, 28 IVL from 26 patients (Cases 4 and 24 were recurrences of 3 and 17, respectively) were identified and available H&E slides (median: 1 slide, range: 1–17 slides) were reviewed by 4 pathologists (Z.O., E.O., P.H. and N.B.). The following microscopic characteristics were evaluated: cellularity (cellular or highly cellular), epithelioid morphology, hydropic change, hyalinization, hyaline plaques, vascularity and presence of adipose tissue, nuclear atypia, mitotic activity (number per 10 high power fields), and infarct-type necrosis. IVL with significantly more cellularity than the adjacent myometrium was described as “cellular”, while those with cellularity similar to that seen in endometrial stromal tumors were further characterized as “highly cellular”. IVL with features similar to those of uterine and extrauterine angioleiomyoma (numerous evenly distributed vasculature (capillary), venous (thick arteriole-like) or cavernous (widened) surrounded by smooth muscle cells) was classified as “vascular”[25,26]. The vasculature was considered as “usual” type when vessels were variably sized with at least some prominent thick-walled large blood vessels. In addition, tumor vessels were assessed for hyalinization, myxoid change, and thrombi. The extent of morphological features was scored as minimal (<5%), focal (5–24%) or diffuse (>25%). For classification purposes, only when a specific morphology was at least focally present, the tumor was assigned to that category. Clinical and demographic information were retrieved from medical records or pathology reports. Aggressive clinical behavior was defined as presence of tumor outside the uterus or recurrence.

Immunohistochemistry (IHC) was performed on the Leica Bond automated staining platform with appropriate positive and negative controls stained in parallel. Citrate or EDTA antigen retrievals were done using the Leica Biosystems Refine Detection Kit. The following antibodies were used: phosphorylated Rb (Cell Signaling Technology, #9308 clone s807/811, 1:100, citrate retrieval, presence of cytoplasmic or nuclear staining), SOX10 (Cell Marque, #CM383A-76 Polyclonal,1:50, citrate retrieval, extent of nuclear staining), SDHB (Abcam, #14714 clone 21A11AE7,1:1000, EDTA retrieval, lack of cytoplasmic staining), p16 (Roche, #725–4713, 1:4, EDTA retrieval), Cyclin D1 (Leica, #PA0046, 1:1, EDTA retrieval, extent of nuclear staining), SMARCB1 (BD Bioscience, #612110, 1:100, EDTA retrieval, lack of nuclear staining), Carbonic Anhydrase IX (CAIX) (Cell Marque, #379R, 1:1, Citrate retrieval, extent of cytoplasmic/membranous staining) and FH (Thermo Scientific, #PA5–22091, EDTA retrieval, lack of cytoplasmic staining). When assessing extent of staining, a semiquantitative scoring method was used: 0 (no staining), 1 (<5%), 2 (5–24%), 3 (25–50%), and 4 (>50%) in IVL and surrounding non-neoplastic tissue (means between the two compared by Student’s t-test).

DNA isolation and oligonucleotide aCGH assay were performed as previously described after manual dissection of tumor tissue from 5 μm unstained sections of formalin-fixed paraffin embedded tissue[22]. Briefly, DNA was extracted according to the manufacturer’s instructions using Qiagen tissue kit (Qiagen, Chatsworth, CA). Patient and control DNA

labeling, oligonucleotide array hybridization (SurePrint G3 Human CGH 8 × 60K Oligo Microarray Kit with 62 976 probes of 60-mer oligonucleotide per array; Agilent Technologies), post-hybridization wash, image capture, and extraction of signal feature were performed as previously described[27]. Genome-wide copy number variation was analyzed with a threshold value of 6, a cut-off value of 0.25, and a filter of 6 continuous probes in Agilent CytoGenomics 4.0. All nucleotide positions were designated according to the March 2006 Assembly (NCBI36/hg18) in the University of California Santa Cruz (UCSC) Human Genome browser (http://genome.ucsc.edu/). Genome-wide distribution and relative frequency of the chromosomal abnormalities were resulted from the raw data of copy number alterations in cases. Database of Genomic Variants (http://dgv.tcag.ca/dgv) was used to determine known copy number variants[28]. Cancer related genes in the recurrent copy number aberrations were detected using the Cancer Gene Census (https://cancer.sanger.ac.uk/cosmic/census?tier=all#cl_search) tool[29], including Tier 1 (documented activity in cancer with genomic alterations promoting oncogenic transformation) and tier 2 (recently emerging with strong indications of a role in cancer) genes, as well as literature search. Genomic index was calculated as A^2/C (A=total number of alterations, C=number of involved chromosomes)[30–32]. Genomic index scores between IVL with aggressive clinical features and those without were compared by Mann-Whitney U Test. Unsupervised hierarchical cluster analysis was performed based on the presence of recurrent aberrations within the indicated chromosome band detected in at least 4 cases (~ 15%). Of note, aCGH results from 3 cases (5, 6 and 22) had been previously reported[22].

Results

Clinical Data

Twenty-eight IVL from 26 patients with a median age of 45.5 (34 to 82) years at diagnosis were analyzed. Tumors ranged in size from 0.2 (microscopic) to 17 (median 6) cm. Accompanying UL was noted in 18 IVL. At least 5 year-follow up was available for 23 patients with a median of 13 (up to 25) years. Fourteen IVL (12 patients) were associated with extrauterine extension, and 5 with recurrence (3 patients). In two instances IVL was incompletely resected due to anatomic extent of the tumor (Table 1).

Tumor Morphology

The most common morphologies were cellular (n=15, including 4 highly cellular), usual (n=11), and vascular (n=5, including 3 cellular IVL, showing both vascular and cellular features). There were 2 IVL with adipose tissue and 2 with epithelioid morphology, all of them cellular (Table 2). Hydropic change was detected in 11 (7 focal), hyalinization in 10 (4 focal), and both in 7 while hyaline plaques were present in 7 IVL (4 minimal, 1 focal) (Figure 1). Twenty-three IVL had usual vasculature with prominent thick-walled large caliber vessels and cleft like spaces. Vascular IVL (n=5) included capillary predominant (n=2) and arteriole-like (n=3) vasculature (Figure 2). Ten IVL had vascular hyalinization (6 focal), Myxoid change within vessel walls was seen in 2 cases, one with extensive thrombi, hemorrhage and hemosiderin deposition (Figure 1). All associated UL available for review (15/18) had overlapping morphology with the accompanying IVL (7 usual, 7 cellular

including 1 epithelioid) except for 1 highly cellular IVL with usual UL (Table 2). Tumors were characterized by bland cytologic features and up to 2 mitoses/10 high power fields.

Immunohistochemistry

All IVL showed increased expression of either p16 or Cyclin D1 (mean scores 2.18 and 1.79) in comparison to surrounding non-neoplastic tissue (mean scores 0.58 and 0.88) ($p<0,0001$ and $p=0.0032$). Extensive (score ≥3) p16 or Cyclin D1 staining was detected in 10 (38%) and 6 (21%) IVL, respectively, with 3 (11%) showing positivity for both (Table 2). All but one IVL (Case 23), as well as their surrounding normal tissue displayed cytoplasmic phosphorylated-Rb staining with background nerve and endometrial cells showing nuclear localization. SMARCB1 and FH expression was preserved in all IVL while SOX10 or Carbonic Anhydrase IX were lacking in all.

Molecular Alterations

The most frequently involved chromosome arms were 1p (39%), 22q (36%), 2q (29%), 1q (25%), 13q (21%), 14q (21%), 3q (18%) and 10q (18%) (Figure 3, Table 3, Supplementary Figures 1 and 2) with a median genomic index of 4.75 (0 to 19.3) (Table 2). Putative affected genes in these regions include *MEAF6* (1p), *SMARCB1* and *DEPDC5* (22q), *RB1* (13q), *RAD51B* (14q), *KAT6B* and *FAM22B* (10q), in addition to other genes of potential pathogenetic implications on chromosomes 8q (*PLAG1*), 17p (*MYOCD*) and Xq (*COL4A5/6*), among others (Table 3.)

Three molecular groups were identified based on unsupervised hierarchical clustering analysis of the recurrent copy number alterations, which were predominantly deletions: Group 1 (n=8) and 2 (n=5) with associated del(22q) (most common shared alteration) and group 3 (n=5) with del(10q) (Figure 4, Supplementary Table 1). All three groups had alterations of 1p (n=10) and 1q (n=6). The remaining cases had either non-specific aberrations (n=4) or no alterations (n=6) detected.

Groups 1 and 2 (n=13) showed common alterations involving 22q (n=8), 14q (n=6), 8q (n=2), 9q (n=2), 15q (n=2), 18q (n=2) and Xp (n=2), which were not detected in Group 3. Other specific alterations only present in Group 1 included 2q (n=7), 13q (n=6), 5q (n=4). All IVL in Group 3 (n=5) had del(10q). Non-specific, but amplification-predominant alterations were seen in 5 IVL, including 2 cases with del(22q) that did not have the commonly accompanied alterations present in Groups 1 or 2. Median genomic index scores of Groups 1–3 and those with non-specific and no alterations were 10.2, 3, 2, 7.15, and 0, respectively.

Integrated Clinicopathological and Molecular Characteristics

Cellular appearance (n=15, 54%) was the most frequent morphology and present in all molecular groups. Only 1 cellular (also epithelioid) IVL had no alterations detected. Chromosomal abnormalities in cellular IVL involved 1p (n=9, 6 with 1q, 2 with 10q, 3 with 8q), 1q (n=6), 10q (n=5), 8q (n=4) that were not seen in IVL with usual cellularity except for those with alterations in 1p (n=3, none with accompanying 1q or 8q). Among these, 3 of the 4 highly cellular IVL had alterations of 1p and 1q (2 with del(10q)) and the other had

amplifications of 1q and 8q. Two cellular IVL in Group 3 with del(10q) had adipose tissue (Figure 1). There were no other unique aberrations associated with a specific morphology.

Among the three molecular IVL groups, only tumors in Group 3 had distinct morphological features. All 5 IVL in this group were cellular (2 highly cellular), with hyaline plaques present in 4, adipose tissue in 2, focal to extensive hyalinized vessels with minimal to focal background hyalinization in 2 (Figure 1, Table 2). Three IVL outside Group 3 also had hyaline plaques, including one hypocellular IVL with extensively hyalinized vessels and no detectable copy number alterations (Case 24, recurrence of Case 17 in Group 3, Figure 1)). The other two cases with hyaline plaques had non-specific aberrations (1 highly cellular, 1 usual). The only IVL in Group 3 (Case 15) without hyaline plaques or hyalinized vessels had thrombi and myxoid change in large vessels with associated hemorrhage and hemosiderin deposition, a combination of features not found in any other IVL in our series. No clear correlation was observed between morphologic features and other molecular groups. Among the 6 IVL with no aberrations, 3 were hypocellular due to extensive hyalinization (2 usual, 1 vascular) while the other 3 included 1 usual IVL with focal hyalinization, 1 cellular and epithelioid, and 1 vascular IVL.

Immunohistochemical stains were selected based on vasculotropism of IVL (CAIX) as well as recurrent molecular alterations (all the other immunostains). RB (13q), Cyclin D1 and p16 were analyzed given the frequent deletion of 13q (*RB1*) and regulatory pathway of RB. While cytoplasmic phosphorylated-Rb staining was detected in all but one IVL with hyalinization (Case 23), there was no correlation between p16 or Cyclin D1 expression and tumor morphology, molecular groups or genomic index scores. SMARCB1 (22q), SDHB (1p), and FH (1q) expression was retained. SOX10 (22q) and CAIX were negative in all IVL.

Aggressive clinical behavior was noted in 14 IVL (all with extrauterine extension and 5 associated with recurrences), with no significant differences in morphology, p16 and Cyclin D1 expression, molecular groups (Table 2), or genomic index scores (5.15 vs 3.5) compared to IVL without aggressive behavior.

The majority of IVL associated with recurrence showed vascular morphology (4 out of 5) (Figure 2). The distribution of recurrences between molecular groups was as follows: Group 1 (n=2), Group 2 (n=1), Group 3 (n=1) and no alterations (n=1). Alterations in 8q were detected in 3 of the recurrent IVL. One patient (Cases 3 and 4) had pelvic recurrence excised 5 years later. Morphologically both tumors showed hydropic change and were focally hyalinized. The recurrence had increased numbers of interspersed capillary lumens. The recurrence showed all the aberrations observed in primary tumor : deletions in 1p,2p,2q,13q,14q, 22q and amplifications in 8p and 8q and in addition, a new amplification in 2p (480 kb downstream to 2p deletion) and a deletion in 5q (Supplementary Table 1). Another recurrent IVL (Case 13, primary not available) had a deletion of 8q upstream to the aforementioned 8q amplification with deletions in 1p, 18q, 19q and 22q. Another patient had a recurrence 4 months later (cases 17 and 24). The primary tumor was hydropic with prominent hyalinized arteriole-like vessels and had del(10q) while the recurrence was highly vascular with arteriole-like hyalinized vessels, limited cellularity and no genomic alterations.

Although not considered a recurrence, it is interesting to note that another patient (Case 15) with del(10q) showing large vessels with extensive thrombi, hemorrhage, myxoid change and hemosiderin deposition without prior treatment, presented 10 years later with a left palm mass that was histologically a thrombosed vein.

Pelvic lymph node involvement was noted in two IVL (Cases 14 and 20), both highly cellular. Case 14 also had adipose tissue and was found to have deletions in 1p,1q, 3q and 10q. Case 20 had parametrial and pelvic soft tissue extension and showed amplification of both 8p and 8q.

Discussion

IVL is a rare uterine neoplasm with less than 400 reports in the literature[33–35], and is defined as an intravenous endothelium-coated benign smooth muscle proliferation outside the confines of a leiomyoma. The histologic features of IVL overlap with those of typical leiomyoma and leiomyoma variants, including cellular, epithelioid, and hydropic variants, leiomyoma with bizarre nuclei, lipoleiomyoma, and other less common variants[1,36]. The morphology of IVL may also overlap with other uterine mesenchymal tumors, most commonly hydropic leiomyoma, highly cellular leiomyoma with seedling leiomyomas, low-grade endometrial stromal sarcoma and rarely with ULMS if the latter shows a prominent intravascular component[37–39]. However, the diagnosis in most cases is straightforward, as low-grade endometrial stromal sarcoma typically presents as a multinodular mass involving the endo-myometrium with tongue-like pattern of myometrial invasion, and is histologically composed of relatively uniform endometrial stromal cells with spiral arterioles[40]. Leiomyosarcoma with prominent vascular involvement shows high-grade cytology, brisk mitotic activity and/or tumor cell necrosis, features that are lacking in IVL[39]. Leiomyoma with hydropic change and highly cellular leiomyoma with seedling leiomyomas may closely mimic the appearance of IVL at low power magnification[37,41]. IVL may extend outside the uterus, into pelvic vessels, inferior vena cava, the right heart, or may even involve the pulmonary vessels, and it has a recurrence rate of approximately 10%[2–5]. Few studies have been conducted to investigate the molecular and immunohistochemical profile of IVL. In this study, we analyzed 28 such tumors by aCGH and immunohistochemistry to further understand their clinicopathologic and genomic characteristics.

Frequent Molecular Alterations in IVL

IVL has previously been characterized by der(14)t(12;14), a unique cytogenetic alteration detected by karyotyping[42]. This aberration is considered different than the balanced t(12;14) with *HMGA2-RAD51B* fusion seen in UL. Despite their identical breakpoints, der(14)t(12;14) cytogenetically corresponds to an unbalanced translocation which has two normal chromosome 12, and one normal and one derivative chromosome 14 with a deleted portion of 14q which is presumably replaced by 12q. Therefore, it has been proposed that the presence of an extra copy of 12q and/or loss of 14q may be critical genetic events leading to intravascular proliferation. There is only one reported IVL with a der(14)t(12;14) characterized by karyotyping and FISH that was subsequently analyzed by microarray[23]. It harbored complex chromosome alterations including del(14q) involving *RAD51B*,

surprisingly without an increase in 12q copy number, as well as small deletions in 1p and 1q among others. Interestingly, presence of ring chromosome 1 (presumed to have copy number alterations in 1p, 1q or both) is reported to be a concurrent change in UL with a t(12;14)[43]. This observation parallels the findings in our IVL with del(14q) (n=6, 5 involving *RAD51B*), 4 with an accompanying 1p deletion (1 tumor also had 1q gain) and none with 12q alterations. In addition to del(14q) (21%), other most common alterations involved 1p (39%), 22q (36%), 2q (29%), 1q (25%), 13q (21%), 3q (18%), and 10q (18%) (Figure 3, Table 3), which is similar to a previously reported series[22].

Immunohistochemical Characterization of IVL

Deregulation of the Rb/E2F pathway has been identified in a wide range of tumors by altering expression of genes involved in angiogenesis, epithelial-mesenchymal transition, invasion, and migration[44]. Hyperphosphorylation of Rb promotes tumorigenesis by blocking apoptosis and stimulating proliferation and invasion, which is regulated by Cyclin D1 and p16 proteins[45,46]. In many cancer types alterations leading to increased Rb phosphorylation are more common than Rb mutations and therefore, targeted therapies are designed to inhibit the activity of cyclin dependent kinases toward Rb[47]. In this series, one of the most frequent alterations was del(13q) (*RB1*) (Table 3, Supplementary Table 1). Ser807 is a site phosphorylated by Cyclin D1/CDK4 that is shown to be important for hyperphosphorylation of Rb and its nuclear export resulting in its inactivation[48]. In this study, all but one IVL, which was hypocellular, displayed cytoplasmic phosphorylated-Rb localization, suggesting that nuclear export may be the mechanism for Rb inactivation.

IVL Molecular Groups *vs* UL Subtypes

IVL were grouped by hierarchical clustering of recurrent aberrations to further correlate the concurrent alterations amongst each other as well as with clinicopathologic findings (Figure 4, Table 2). Three molecular groups were identified, while the rest had non-specific/no alterations. The two most common characteristic changes in Groups 1 (n=8, 29%) and 2 (n=5, 18%) were del(22q) (n=8) and del(14q) (n=6), whereas all IVL in Group 3 had a distinct profile of del(10q) (n=5, 18%). Alterations involving 1p (n=10) and 1q (n=6) were seen in all three groups. Based on these results, Group 1 can be considered the genomically unstable counterpart of Group 2 (median genomic index=10.2 vs 3) given multiple unique alterations in Group 1, including changes in 2q, 13q, 5q, besides the common alterations seen in both groups. Four IVL (14%) had copy number changes that could not be categorized into any specific group, showing predominantly amplifications, in contrast to Groups 1–3 showing predominantly deletions. Six (21%) IVL had no alterations detected (3 of them with limited tumor cellularity due to extensive hyalinization).

The three genomic groups described herein are comparable to the molecular subtypes described in UL, despite technical limitations related to detection of point mutations and balanced rearrangements by aCGH. In a study by Mehine *et al.* with selective enrichment of otherwise rare del(22q) UL (20/94), four UL subtypes were identified integrating both gene expression data and genomic alterations: *HMGA2* (12q), *MED12* (Xq), *FH* (1q), and *COL4A5-COL4A6* (Xq))[18]. Among these, *HMGA2* UL usually had t(12;14) with *RAD51B* (14q) as the preferential translocation partner, whereas *MED12* UL showed up-regulation of

RAD51B expression. Nineteen out of 20 del(22q) UL clustered in either *HMGA2* (12) or *COL4A5/6* (7) subtypes. In our series, 8 out of 10 del(22q) IVL clustered together with IVL having del(14q) (n=6, 5 involving R*AD51B*) and del (Xq) (n=2, 1 involving *COL4A5/6*) in Groups 1 and 2 (Figure 4, Supplementary Table 1). Although the number of cases is limited in both studies, it is worth noting that both IVL and UL with del(22q) appeared to have overlapping molecular features with tumors having genomic alterations in *HMGA2/RAD51B* or *COL4A5/6* based on their preferential clustering with such tumors. In addition, UL with 22q alterations were further characterized by studying minimally deleted regions (27,111,559–33,871,686) and a UL with 22q translocation. *DEPDC5* (with 5 UL showing a "second hit" mutation in this gene) and *SMARCB1* were identified as the two putative target genes involved in 22q UL. Not only the minimally deleted 22q region reported in UL had a 4 Mb overlap with one reported herein (27,111,559–33,871,686 *vs* 25,351,942–31,211,236, respectively, Table 3), but also both *DEPDC5* and *SMARCB1* were either involved by or within 1 Mb of the altered region in 9 out of 10 IVL with del(22q). Taken together, IVL with del(22q) and del(14q) are common and tend to cluster together in same molecular groups, which appear to share similar molecular characteristics with their UL counterparts. Of note, none of the del(14q) IVL in our study had a del(10q), however, the aforementioned IVL described elsewhere[23] with der(14) with del(14q) also had a del(10q).

Group 3 IVL (18%) were characterized by del(10q). Alterations involving 10q have rarely been reported in UL (2%)[49], although specifically del(10q) are more commonly observed in ULMS than UL[50]. *KAT6B*, a rare tumor suppressor gene with homozygous deletions in multiple cancer types[51,52], is described as the candidate 10q gene in UL in a study analyzing the rare t(10;17) event[53] and a similar t(10;17), resulting in a *KAT6B-KANSL1* fusion detected in a retroperitoneal and more recently in a uterine leiomyoma[54,55]. This fusion is located at exon 3 (histone binding domain) of *KAT6B* without involvement of the downstream functional domains (histone acetylation and transcriptional activation), implicating *KAT6B* loss of function. Furthermore, germline mutations in both *KAT6B* and *MED12* are associated with Ohdo Syndrome, a heterogenous group of disorders with intellectual disability and craniofacial anomalies[56]. Therefore, previous studies suggested that *MED12* and *KAT6B* are functionally related, both encoding for chromatin-modifying enzymes and implicated in same diseases with mutations at germline and somatic levels[56,57]. Interestingly, in our series all del(10q) IVL in Group 3 (5) involved K*AT6B* and none clustered together with del(22q), which may be parallel to UL groups with only 1 out of 20 del(22q) clustering with the *MED12* subtype[18].

Overall, our IVL molecular groups had overlapping characteristics with UL subtypes, albeit the latter has been studied more comprehensively due to integration of RNA data. Commonly altered chromosome regions in IVL (Table 3) are less frequently involved in UL except for 14q (*RAD51B*). However, despite 14q being a frequently altered region in IVL and UL, the unbalanced nature of this aberration remains unique to IVL. The paucity of the common IVL alterations in UL may provide insight into the fact that only a very small subset of women with UL have accompanying IVL. Analysis of additional IVL along with RNA expression and fusion transcript data may help further characterize IVL molecular groups and their correlations with UL subtypes.

Alterations in IVL *vs* other UL Variants with Unusual Clinical Behavior and Soft Tissue LM

Benign metastasizing leiomyoma (BML) and disseminated peritoneal leiomyomatosis (DPL) are considered other unusual leiomyoma variants. Alterations involving 1p, 2q, 3q, 7p, 11q, 19q, 22q have been reported in BML[58–60]. A case report of a cellular DPL after morcellation of a hydropic and hyalinized UL showed r(1), del(3q), del(9q) and t(12;14) on karyotype with 1p, 1q, 2p 2q 3q, 14q loss and 11p gain on microarray, in parallel to the aforementioned associations of IVL with der(14) and its relationship to r(1) and del(14q). In a study analyzing 8 soft tissue LM (all female patients with retroperitoneal/pelvic tumors), 3 had 12q alterations (inv(12); t(12;14); der(12) and del(14q) as well as −22), 3 had 8q (ins(8)(p23q12q22), t(8;14)(q13;q24), t(8;19)(q12;q13)), 1 had del(7) and del(14q), 1 had t(3;11) and add(11q). Tumors with 8q alterations showed PLAG1 involvement[61], which was also amplified in 3 IVL with aggressive behavior in our series. These rare subsets of leiomyomas occurring outside the uterus have recurrent alterations similar to those seen in IVL, which are rarely seen in UL.

Alterations in Cellular IVL *vs* Cellular UL and Endometrial Stromal Sarcoma (ESS)

Cellular UL has been associated with del(1p) and t(10;17)[19,53,62]. In particular, a study analyzing 9 UL with del(1p) and other aberrations including loss of chromosomes 19 and/or 22, and less commonly 10q, 13q and 14, reported 6 of them being cellular[62]. In another study, gene expression analysis demonstrated clustering of IVL with ULMS and cellular/atypical UL with 1p deletion, rather than with myometrium, usual or plexiform UL[23]. In the current series, chromosomal abnormalities in cellular IVL involved 1p, 1q, 8q and 10q. Among these, 3 of the 4 highly cellular IVL had alterations of 1p and 1q (2 with del(10q)) and the other had amplifications of 8q and 8q. Although the copy number alterations reported herein are mostly Mb-sized larger genomic regions, it is interesting to note that some of the recurrent alterations in cellular IVL involve genes altered in ESS genomic rearrangements including *MEAF6* (1p) and *FAM22B (10q)* (Table 3).

Vascular IVL *vs* Angioleiomyoma

Angioleiomyoma (angiomyoma, vascular leiomyoma) is a benign soft tissue tumor with thick-walled vessels formed by proliferating smooth muscle cells, and vascular channels, which can rarely occur in the uterus[26]. Deletions in 6p[63] and 13q have been described in soft tissue tumors.[64]. A microarray analysis of these tumors showed recurrent 22q loss as the most common alteration (22%)[65]. Cytogenetic analysis of a uterine angioleiomyoma showed a complex karyotype with abnormalities involving 2p, 2q, 5q, 11p, 20q and Xp including inv(2)(p15q13)[66]. None of these alterations are common in UL, however, they mostly overlap with IVL reported herein including those with vascular morphology, albeit not specific to this morphologic subtype.

IVL with del(22q) and del(14q) *vs* Gastrointestinal Stromal Tumors

Loss of 22q is one of the most frequent alterations in IVL, identified in 36% of tumors in our series and in two-thirds of tumors in a previously published smaller series[22]. In addition, we observed loss of 14q in 21% of IVL. Interestingly, losses involving chromosomes 22q and 14q have also been frequently found in gastrointestinal stromal tumors (GIST)[67–70], and it

has been suggested that these alterations play an important role in early stages of GIST tumorigenesis[70]. In addition, allelic losses at 22q in GIST were found to be associated with high mitotic activity and disease recurrence[71]. We observed loss of 22q in 6 of 14 IVL with extrauterine extension and in 3 of 5 recurrent tumors, suggesting the possibility of a similar association. From a potential diagnostic aspect, although karyotyping or array CGH are not typically performed as part of the routine diagnostic work-up, the overlap in tumor genomic profiles between GIST and IVL should also be taken into account when encountering spindle cell tumors at unusual peritoneal locations.

IVL *vs* Smooth Muscle Tumors of Uncertain Malignant Potential (STUMP)

Although IVL is histologically benign, our series shows molecular features that parallel those reported in STUMP[72]. An array genomic hybridization analysis of 29 STUMP showed alterations involving 22q (70%), 13q (50%), 11p (50%), 6q (50%), 3q (50%) and 1q (60%)[31]. In addition, a recent series by Croce *et al.* of 77 uterine smooth muscle tumors with a mean follow up of 63.6 months identified a genomic index cut off score of 10 as a predictor of recurrence and a criterion for "molecular leiomyosarcoma"[32]. Among stage 1 "molecular leiomyosarcomas" in that study, poor prognostic markers included genomic index cut-off score of 35, 5p gain, 13q loss involving *RB1* and 17p gain involving *MYOCD*, the latter promoting smooth muscle differentiation and cell migration[73]. Alterations of 22q (36%), 13q (21%), 6q (14%), 3q (18%) and 1q (25%) (Table 3) were overlapping recurrent aberrations detected in IVL in our series (predominantly in Group 1), although less frequent than those previously reported in STUMP. Six (21%) IVL had a genomic index of ≥10 (all <35), also mostly in Group 1 (4 of them). However, there was no association between genomic index (with or without specific cut off of 10) and clinically aggressive behavior in IVL. It is interesting to note that Case 5 (Group 1) had the highest genomic index (19.3) and would have qualified as "molecular leiomyosarcoma" with two poor prognostic factors (5p gain and 13q loss) based on the aforementioned study. However, it had usual morphology, was confined to the uterus, and the patient had no recurrence after 6 years. Therefore, especially in the setting of leiomyoma variants, results of genomic analysis should be interpreted carefully in correlation with the pathologic findings. That said, the majority of the tumors in our series showed simple genomic profiles (genomic index ranging from 0 to 19.3; mean 5.9), significantly different from the previously reported genomic profiles of leiomyosarcomas (with genomic index up to 180, and mean 51.8)[32]. Future studies - using other techniques (mutation and gene expression analyses) – may uncover additional genomic alterations not detectable by aCGH (mutations or balanced rearrangements) that may be responsible for the intravascular tumor location and the resulting aggressive clinical behavior in some patients.

Conclusion

Herein, we describe three genomic groups of IVL, in which del(10q) and del(22q) were mutually exclusive with overlap in other aberrations including del(1p), comparable to molecular subtypes reported in UL. The described recurrent chromosome alterations included target genes reported in other uterine and extrauterine mesenchymal tumors with unique histologic characteristics particularly for cellular, vascular and Group 3 tumors with

del(10q). The previously proposed genomic index for uterine smooth muscle tumors did not predict a malignant course in our series and there was no specific molecular alteration associated with aggressive behavior. Our molecular and immunohistochemical findings suggest involvement of the Rb pathway in the pathogenesis of a subset of IVL. Currently, this is the largest series correlating histological, immunohistochemical, and molecular characteristics of IVL. While our molecular analysis is limited to array CGH, these data may serve as a platform for future studies - including additional protein expression analysis and next generation sequencing – on IVL and paired adjacent leiomyomas to further explore their potential pathogenetic relationship and elucidate the critical steps of IVL tumorigenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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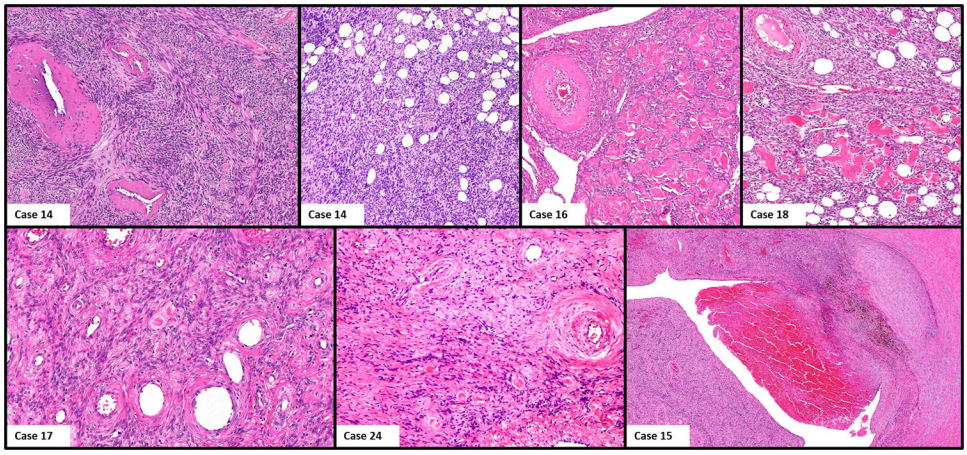

Figure 1. Morphological characteristics of molecular group 3 IVL (Cases 14–18) and Case 24 (recurrence of Case 17).

Note the hyaline plaques (Cases 14, 16–18, and 24) and hyalinized vessels (Cases 14, 16, 17 and 24), adipose tissue (Cases 14 and 18), and large vessel with thrombosis, myxoid change and hemorrhage (Case 15). All these IVL are cellular except for Case 24, which is extensively hyalinized and cellularity is difficult to assess.

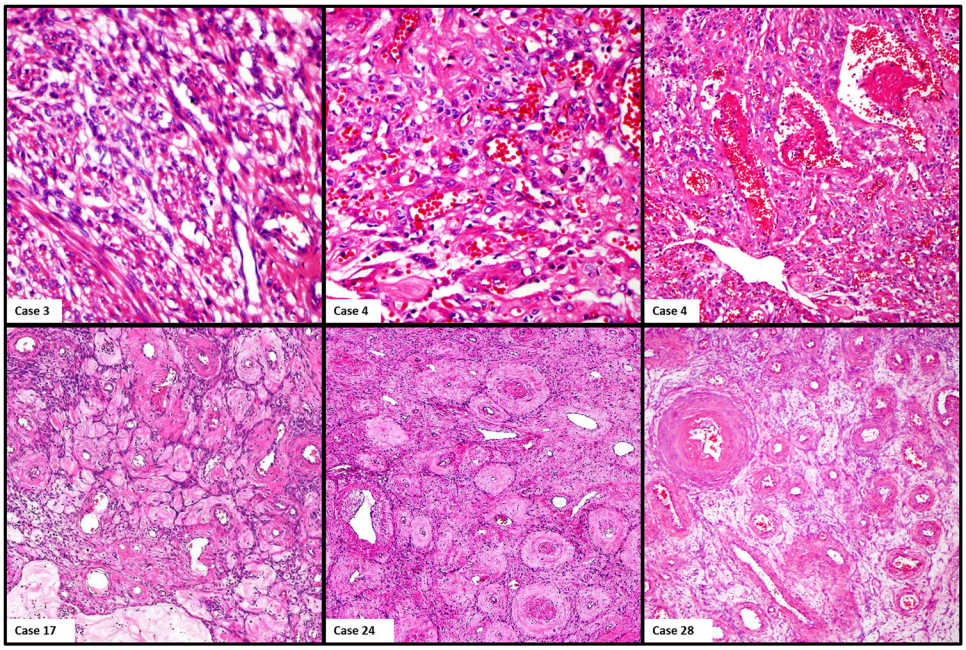

Figure 2. Morphological characteristics of vascular IVL.

Cases 3 and 4 (recurrence of Case 3) have capillary-predominant, whereas Cases 17, 24 (recurrence of Case 17), and 28 have arteriole-like vasculature. In addition, Case 4 has myopericytoma-like vessels with minimal thrombi, which were not seen in Case 3.

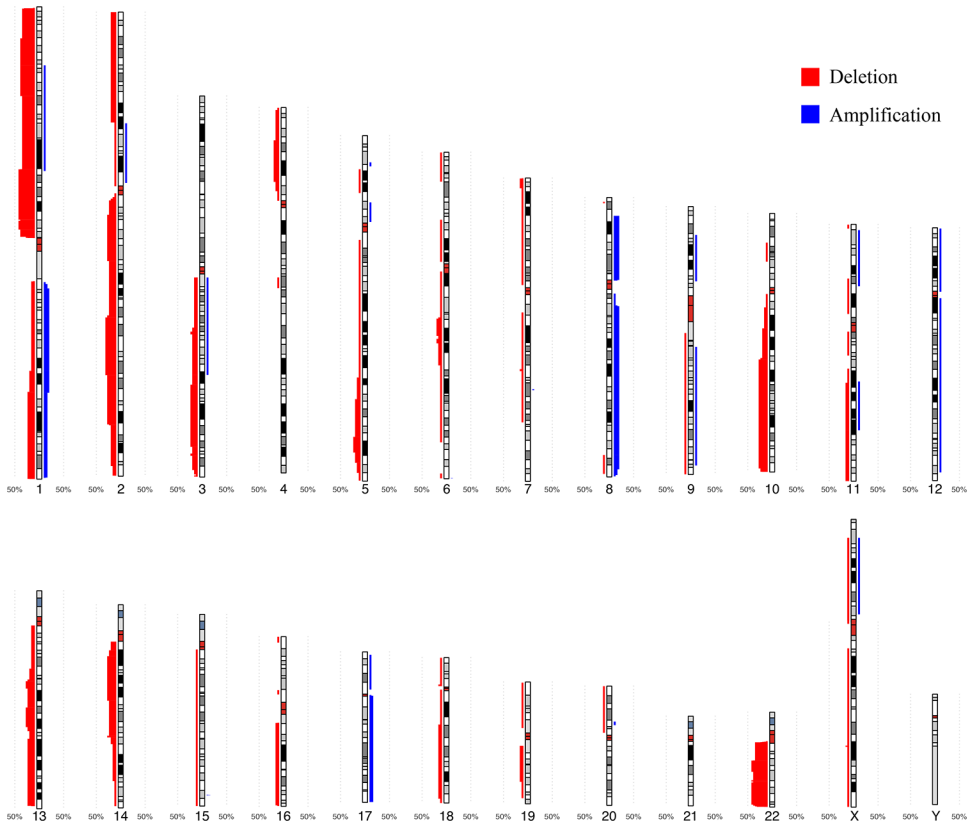

Figure 3.
Chromosome view of copy number alterations.

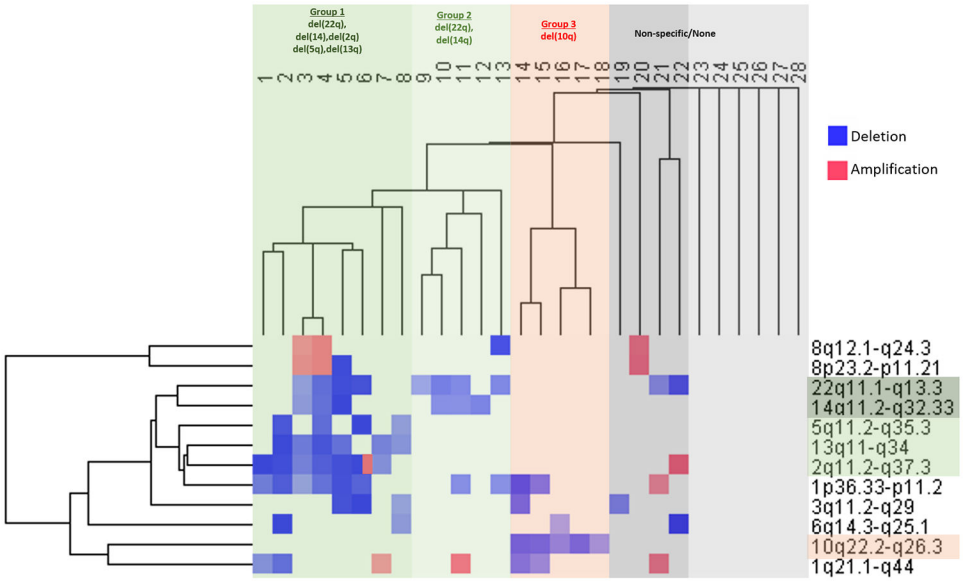

Figure 4. Molecular groups.

Unsupervised hierarchical clustering of the recurrent copy number alterations.

Table 1.

Clinical characteristics

	Case	Age (y)	Size	Other Findings	Extrauterine Extension	Follow up (y)	Outcome
Group 1	1	44	1.1 kg	None	YES (Extrauterine vascular, bladder wall)	N/A	N/A (At the time of surgery: incomplete resection due to bladder wall involvement)
	2	39	12 cm	Adenomyosis, Ovary: Mature cystic teratoma	NO	5	NED
	3	41	5 cm	Leiomyoma	YES (Extrauterine vascular)	23	Recurrence in 5 years (see Case 4)
	4	46	11.5 cm	None	YES (Pelvic wall)	18	NED (Recurrence of Case 3)
	5	42	2.5 cm	Leiomyomata, adenomyosis	NO	6	NED
	6	49	8 cm	Leiomyoma, adenomyosis	YES (Extrauterine vascular)	20	NED
	7	51	6 cm	Leiomyomata	NO	8	NED
	8	57	2 cm	Leiomyomata	NO	18	NED
Group 2	9	50	10 cm	Leiomyomata	NO	18	NED
	10	42	0.5–5 cm	Gastric carcinoma	YES (Extrauterine vascular)	17	NED
	11	43	N/A	Leiomyoma	NO	17	NED
	12	34	0.2–5 cm	N/A (myomectomy)	NO	11	NED
	13	44	14 cm	None	YES (Ureter, right retroperitoneum, vena cava, paraspinous muscle)	18	NED (This case is a recurrence after prior hysterectomy with adnexal involvement)
Group 3	14	82	11 cm	Leiomyomata, Right Kidney: Angiomyolipoma	YES (Left external iliac artery, bilateral ovaries, lymph node)	6	NED
	15	50	4.5 cm	Leiomyomata, benign mesothelial cyst in peritoneum	YES (Extrauterine vascular, parametrium)	10	NED, 1.5 cm pseudoaneurysm in left palm (vein with organizing thrombus)
	16	47	8.5 cm	Leiomyoma, adenomyosis, Ovary: Extensive endometriosis	YES (Extrauterine vascular, pelvic wall)	N/A	N/A
	17	40	Right Ovary: 17 cm Left Ovary: 3.5 cm Uterus: 0.3–0.5 cm	Pelvic floor vascular malformation	YES (Bilateral ovaries)	6.5	Recurrence in 4 months (see Case 24)
	18	57	2 cm	Adenomyosis	NO	9	NED
Non-specific	19	46	0.5–4 cm	Leiomyomata, endometrial polyp	NO	7	NED
	20	45	6.5 cm	Leiomyomata	YES (Extrauterine vascular, parametrium, pelvic wall, lymph node)	5	NED
	21	40	9 cm	Leiomyoma	YES (Extrauterine vascular, parametrium)	23	NED

	Case	Age (y)	Size	Other Findings	Extrauterine Extension	Follow up (y)	Outcome
	22	64	1.2 cm	Leiomyoma	NO	5	Died of other disease (no recurrence)
None	23	70	1 cm	None	NO	20	NED
	24	41	17 cm	Pelvic floor vascular malformation	YES (Inferior Vena Cava)	6	3 years follow up: Metastasis to lung (multiple <1 cm lesions), bilateral pelvic and inguinal lymph nodes, left pelvic mass (6.3 cm) with bladder wall involvement (Recurrence of Case 17) 6 years follow up: Alive with disease
	25	40	N/A	Leiomyoma	NO	13	NED
	26	40	0.2–0.6 cm	Leiomyomata	NO	2 months	NED
	27	64	1.5 cm	Leiomyomata, adenomyosis, bilateral ovaries with surface papillomatosis	NO	25	NED
	28	56	11 cm	Leiomyomata	YES (Extrauterine vascular, left broad ligament)	14	NED

NED: No evidence of disease, N/A: Not applicable

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Table 2.

Morphologic, Clinical, Genomic and Immunohistochemical Characteristics

	Case	Morphology IVL	Morphology UL	Aggressive Features	Alt	Chr	GI	p16	Cyclin D1
Group 1	1	Cellular, epithelioid	N/A	YES (EUE)	7	5	9.8	2	4
	2	Cellular, focal hydropic, minimal hyalinization	N/A	NO	7	6	8.2	1	1
	3	Cellular, focal vascular (capillary), focal hydropic, focal hyalinization, minimal hyalinized vessels	NAR	YES (EUE, R)	8	6	10.6	2	1
	4	Cellular, vascular (capillary), focal hyalinization, focal hydropic, focal myopericytoma like hyalinized vessels with minimal thrombi and hemosiderin	N/A	YES (EUE, R)	10	7	14.3	4	2
	5	Usual	Usual	NO	17	15	19.3	1	2
	6	Usual, hydropic, minimal hyalinization	NAR	YES (EUE)	14	11	17.8	3	1
	7	Usual	Usual	NO	5	4	6.3	2	2
	8	Usual, hyalinization, focal hydropic, focal hyalinized vessels	Usual	NO	4	4	4	2	1
Group 2	9	Cellular, hyalinization, focal hydropic, focal hyalinized vessels	Cellular, hyalinization, focal hydropic	NO	3	3	3	2	3
	10	Usual, focal hydropic, minimal hyalinization	N/A	YES (EUE)	3	3	3	2	2
	11	Highly cellular	Highly cellular	NO	8	6	10.6	3	1
	12	Usual, hydropic, hyalinization, focal hyalinized vessels	N/A	NO	1	1	1	4	4
	13	Cellular, minimal hyalinization	N/A	YES (EUE, R)	5	5	5	1	1
Group 3	14	Highly cellular, minimal hyaline plaques, minimal adipose tissue, focal hyalinized vessels	Highly cellular	YES (EUE)	10	8	12.5	3	4
	15	Highly cellular, large vessels with thrombosis, myxoid change, extensive hemorrhage and hemosiderin	Usual, hyalinized	YES (EUE)	4	3	5.3	2	1
	16	Cellular, hyaline plaques, minimal hydropic, minimal hyalinized vessels	Cellular, hyaline plaques, minimal hydropic	YES (EUE)	2	2	2	2	1
	17	Cellular, vascular (arteriole-like), minimal hyaline plaques, hydropic, focal hyalinization, hyalinized vessels, hemosiderin	N/A	YES (EUE, R)	1	1	1	4	1
	18	Cellular, hyaline plaques, adipose tissue, hemosiderin	N/A	NO	2	2	2	1	1
Nonspecific	19	Usual, minimal hyalinization, minimal hyalinized vessels	Usual	NO	4	2	8	3	2
	20	Highly cellular (palisading schwannian morphology), focal hyaline plaques, minimal hyalinization	Highly cellular, focal hyaline plaques	YES (EUE)	6	3	12	1	1
	21	Cellular, minimal hyalinization, minimal hydropic change, focal myxoid small to medium sized and minimal hyalinized vessels	Cellular leiomyoma with hydropic change	YES (EUE)	3	2	4.5	1	1

	Case	Morphology IVL	Morphology UL	Aggressive Features	Alt	Chr	GI	p16	Cyclin D1
	22	Usual, minimal hyaline plaques, hydropic, focal hyalinization, focal hyalinized vessels	Usual, hydropic, focal hyalinized	NO	5	4	6.3	1	1
None	23	Usual, hyalinization, minimal hydropic, hyalinized vessels	N/A	NO	0	0	0	1	1
	24	Vascular (arteriole-like), minimal hyaline plaques, hyalinization, hyalinized vessels	N/A	YES (EUE, R)	0	0	0	3	1
	25	Epithelioid, cellular	Epithelioid, cellular	No	0	0	0	1	4
	26	Usual, minimal hydropic	Usual, hyalinized	NO	0	0	0	1	1
	27	Usual, hyalinized, minimal hydropic, hyalinized vessels	Usual	NO	0	0	0	4	4
	28	Vascular (arteriole-like), focal hydropic change, minimal hyalinization	NAR	YES (EUE)	0	0	0	4	1

N/A: Not applicable, NAR: Not available for review, EUE: Extrauterine extension, R: Recurrence, Alt: Number of alterations, Chr: Number of chromosomes involved, GI: Genomic index (Number of alterations^2/number of chromosomes involved).

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Table 3.

Maximum and minimal overlapping regions in recurrent chromosome aberrations with tumor related genes located in the minimal overlapping regions.

Maximum interval (kb)	Minimum interval (kb)	Relevant Genes	Number of cases (%)	
1p36.33p11.2: 564,424-121,322,377 (120,758)	1p35.2p33: 30,665,772-46,928,430 (16,263)	*LCK, SFPQ, THRAP3, CSF3R, MYCL, MPL, MUTYH, MEAF6*	8 (29%)	11 (39%)
	1p22.3p13.3: 85,239,163-111,475,662 (26,236)	*BCL10, RPL5, TGFBR3, RBM15*	9 (32%)	
	1p13.2p13.1: 111,967,027-116,618,073 (4,651)	*TRIM33, NRAS*	9 (32%)	
1q21.1q44: 143,787,504-247,139,492 (103,352)	1q31.2q32.1: 201,586,748-202,001,197 (414)	*ELF3, FH*	7 (25%)	
2q11.1q37.3: 95,529,039-243,028,452 (147,499)	2q12.2q21.1: 106,120,724-130,333,677 (24,213)	*RGPD3, RANBP2, PAX8, ERCC3*	5 (18%)	8 (29%)
	2q31: 176,959,166-177,055,486 (96)	*HOXD13, HOXD11*	7 (25%)	
	2q37.3: 240,116,052-240,320,953 (205)	-	5 (18%)	
3q11.1q29: 93,575,285-198,154,829 (104,580)	3q21.1q24: 123,390,999-146,012,579 (22,622)	*GATA2, RPN1, CNBP, STAG1, PIK3CB, FOXL2, ATR*	4 (14%)	5 (18%)
	3q13.3q21.1: 121,295,144-123,300,941 (2,006)	-	4 (14%)	
5q11.2q35.3: 54,636,411-180,598,584 (125,962)	5q33.3q34: 157,708,829-165,950,962 (8,242)	*EBF1, PWWP2A*	4 (14%)	
6q11.1q25.1: 62,448,434-151,805,950 (89,358)	6q14.3q16.1: 87,044,032-95,997,166 (8,953)	*EPHA7*	3 (11%)	4 (14%)
	6q16.1q16.3: 97,695,181-99,998,194 (2,303)	-	3 (11%)	
8p23.2p11.1: 2,308,926-43,452,795 (41,144)	8p23.2: 2,308,926-2,965,283 (656)	-	1 (4%)	4 (14%)
	8p23.1p11.21: 9,615,685-43,167,985 (33,552)	*LEPROTL1, WRN, NRG1, NSD3, ANK1, KAT6A, IKBKB, HOOK3*	3 (11%)	
8q11.21q24.3: 50,336,903-145,976,051 (95,639)	8q12.1q24.3: 56,899,737-142,274,992 (85,375)	*PLAG1, CHCHD7, PREX2, NCOA2, HEY1, CNBD1, NBN, RUNX1T1, CDH17, COX6C, PABPC1, UBR5, RSPO2, EIF3E, CSMD3, RAD21, EXT1, MYC, NDRG1, FAM135B*	3 (11%)	4 (14%)
10q11.21q26.3: 42,209,250-135,254,513 (93,045)	10q22.2q26.3: 76,458,215-133,476,839 (57,019)	*KAT6B, GATA3, NUTM2B, LARP4B, BMPR1A, NUTM2D, FAM22B, PTEN, FAS, TLX1, NFKB2, SUFU, NT5C2, VTI1A, TCF7L2, SHTN1, FGFR2, CPEB3, CYP2C8, MGMT, MGEA5*	5 (18%)	
13q11q34: 18,194,544-112,732,368 (94,538)	13q14.2q14.3: 47,559,566-52,307,072 (4,748)	*RB1, CYSLTR2*	5 (18%)	6 (21%)
	13q21.2q21.33: 62,222,639-72,463,336 (10,241)	-	5 (18%)	
	13q22.3q34: 77,509,972-112,586,620 (35,077)	*GPC5, SOX21, ERCC5*	4 (14%)	
14q11.2q32.33: 19,323,579-105,432,573 (86,109)	14q12q22.1: 27,867,557-51,125,450 (23,258)	*ARHGAP5, BAZ1A, NKX2-1, FOXA1*	5 (18%)	6 (21%)

Maximum interval (kb)	Minimum interval (kb)	Relevant Genes	Number of cases (%)	
	14q24.1q24.2: 68,603,041-69,446,436 (843)	*RAD51B*	5 (18%)	
22q11.1q13.33: 15,533,988-51,219,009 (35,685)	22q12.1q12.3: 25,351,942-31,211,236 (5,859)	*MN1, CHEK2, ZNRF3, EWSR1, NF2, SMARCB1, DEPDC5*	9 (32%)	10 (36%)
	22q13.1q13.33: 36,788,138-48,390,822 (11,603)	*ZC3H7B, APOBEC3B, PDGFB, MRTFA, EP300*	9 (32%)	
	22q12.3q13.1: 37,300,872-37,976,764 (676)	-	9 (32%)	