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· 临床病例讨论 ·

1 例腺泡状软组织肉瘤的临床病理特征

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[摘要] 采用光镜及免疫组织化学等方法对1例腺泡状软组织肉瘤(alveolar soft part sarcoma, ASPS)进行临床及病理回顾性分析并综合文献复习。该例患者因右臀部肿物14年入院, CT提示右侧臀大肌内见卵圆形稍低密度肿物, 肿物质软, 切面呈实性, 直径约5 cm。光镜下可见特征性腺泡状结构形成, 肿瘤细胞体积大, 细胞质丰富, 可见嗜酸性细胞质, 核内核仁明显可见。部分区域可见血管内瘤栓形成。免疫组织化学结果示该例肿瘤细胞转录因子E3(transcription factor E3, TFE3), 神经元特异性烯醇化酶(neuron-specific enolase, NSE)阳性; 肌调节蛋白(MyoD1), 肌细胞生成素(Myogenin)细胞质阳性; CD31, 平滑肌肌动蛋白(smooth muscle actin, SMA)在肿瘤细胞中阴性, 但在腺泡状结构间的血管壁为阳性; 结蛋白(Desmin)阴性, Ki-67增殖指数<20%。酶消化后PAS染色示: 细胞质内可见嗜伊红色细颗粒状及红染的棒状结晶, 此为ASPS的特征性改变。半定量RT-PCR结果示: 该例肿瘤表达ASPL-TFE3 1型融合基因mRNA。ASPS多见于青少年和青年, 但多数患者早期出现血道转移, 最终预后欠佳。结合临床和病理学特征及基因诊断, 可作出正确的病理诊断。本例患者于手术切除后2年出现肺多发转移灶及髂骨转移病灶, 后行帕左帕尼(小分子抗血管生成靶向药)治疗后, 肿物明显缩小。目前病情趋于稳定, 但该肿瘤临床生物学行为难以确定, 需对患者进行长期随访。

[关键词] 腺泡状软组织肉瘤; 临床病理特征; 免疫组织化学

A case report of clinicopathological features of acinar soft tissue sarcoma

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Abstract The clinical characteristics and the microscopic and immunohistochemical characteristics of 1 case with alveolar soft part sarcoma (ASPS) were analyzed with a follow-up period and related literature was reviewed. The patient was admitted to the hospital with a right hip mass for 14 years. CT showed that an oval mass with slightly low density was in the right gluteus maximus. The mass was soft and the cut surface was solid. The tumor cells were large in size and had abundant cytoplasm, and nucleus was visible. The results of immunohistochemistry showed

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that tumor cells were positive for MyoD1, Myogenin, NSE, TFE3, and were negative for Desmin, CD31 and SMA with Ki-67 proliferation index less than 20%. But the vessel wall around the tumor cell clusters is positive for CD31 and SMA. PAS staining showed eosinophilic granules and crystals in the cytoplasm of tumor cells. This case expressed ASPL-TFE3 type 1 fusion gene mRNA by RT-PCR. ASPS mostly occurs in adolescents and young people, but most patients have early hematogenous metastasis, the final prognosis is poor. Combined with clinicopathological features and genetic diagnosis, we can make the correct pathological diagnosis of this disease. In this patient, multiple metastatic lesions of the lung and metastasis of the iliac bone occurred two years after surgical resection, and after the treatment with pazulopani (small molecule anti-angiogenic targeted drug), the tumor was significantly reduced. At present, the condition tends to be stable, but the clinical biological behavior of the tumor is difficult to determine and long-term follow-up of the patient is required.

Keywords alveolar soft part sarcoma; clinicopathological features; immunohistochemistry

腺泡状软组织肉瘤(alveolar soft part sarcoma, ASPS)是一种较少见的组织起源尚不明确的软组织恶性肿瘤,多见于青少年,主要累及躯干及四肢^[1]。近年来,在ASPS中发现特异的非平衡性t(X:17)(p11.2;q25)染色体易位,随后又通过反转录-聚合酶链反应技术检测到这种易位产生的ASPL-TFE3融合基因,推测其可能是一个有效的诊断指标。

1 临床资料

患者,男,34岁,发现右臀部肿物14年,2002年行下颌骨骨折手术,2009年行右踝部肌腱手术。入院查体:右下肢麻木,生理反射存在,病理征未引出,右下肢皮肤感觉正常,血运良好。MRI,CT,B超等影像学检查示:右侧臀大肌内低密度样肿物。术中见肿瘤体积为6 cm×5 cm×5 cm,包裹1~2 cm完整肌肉套,剖面灰白色,质地细腻如鱼肉组织,肿瘤周围血供丰富。

1.1 标本处理

常规手术切除标本经4%甲醛固定,常规取材,石蜡包埋,常规HE染色。免疫组织化学采用EnVision二步法,所用抗体转录因子E3(transcription factor E3, TFE3),CD31,结蛋白(Desmin),平滑肌肌动蛋白(smooth muscle actin, SMA),肌调节蛋白(MyoD1),肌细胞生成素(Myogenin),神经元特异性烯醇化酶(neuron-specific enolase, NSE),Ki-67均购自福州迈新生物技术开发有限公司,每次染色设阳性及阴性对照。特殊染色采用PAS染色。采用RT-PCR方法进行ASPL-TFE3融合基因的检测。引物如下:TFE3 exon4 Rev为

5'-CGTTTGATGTTGGGCAGCTCA-3'; TFE3-N exon4 Rev为5'-TGATGGCTGGTGTGGCCACG-3'; ASPL上游引物为5'-AAAGAAGTCCAA-GTCGGGCCA-3'; ASPL-N上游引物为5'-CGGGCCAGGATCCCCAGCAG-3'。

1.2 标本观察及病理诊断

影像学检查示:右侧臀大肌内见卵圆形稍低密度样肿物,大小约56 mm×42 mm,边缘较清楚,增强扫描动脉期病灶明显强化,不均匀,其内见低强化影,延迟扫描病灶仍未见高密度影,病灶内由髂内动脉供血并可见回流血管影。盆腔内未见明显异常(图1)。大体观察示:肿瘤体积约为5 cm×5 cm,边缘附有少量肌肉及梭形皮肤,肿块呈椭圆形,表面呈灰黄色,质软,实性如鱼肉组织,包膜完整(图2)。光镜下可见腺泡状结构,周围由新生血窦包绕,肿瘤细胞体积大,胞质丰富,局部可见嗜酸性胞质,内含伊红颗粒,细胞核较大,呈卵圆形,均一,着色较淡,核内核仁明显,偶见非典型细胞(图3,4),可见血管内瘤栓形成,瘤栓内包含多个肿瘤细胞,核分裂象较多,明显异型(图5)。免疫组织化学示:TFE3胞核阳性(图6),MyoD1细胞质阳性(图7),Myogenin细胞质阳性(图8),NSE细胞质阳性(图9),CD31阴性(图10),SMA阴性(图11)但在血管壁为阳性,Desmin阴性,Ki-67增殖指数<20%。PAS染色示:胞质内含有针状或棒状样结晶体,耐淀粉酶消化(图12)。该例患者样本经第一轮PCR及巢式PCR后,可见100~200 bp之间有条带,为ASPL-TFE3 1型融合基因,长度为138 bp(图13)。病理诊断:(臀部)腺泡状软组织肉瘤。



图1 CT示右侧臀部可见卵圆形稍低密度样肿物(箭头)

Figure 1 CT shows that a small, low-density, oval-shaped mass in the right buttock (arrow)



图2 肉眼观肿物直径约5 cm, 界限清楚, 可见包膜(箭头)

Figure 2 The tumor is approximately 5 cm in diameter with clear boundaries and visible envelopes (arrows)

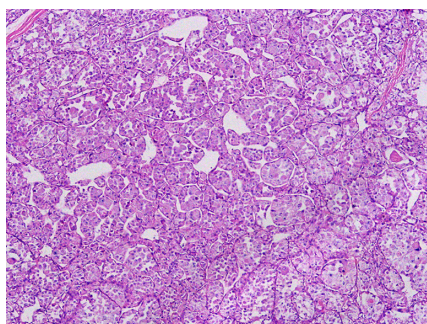


图3 肿瘤细胞呈特征性腺泡状结构(HE, × 100)

Figure 3 Tumor cells show characteristic alveolar structure (HE, × 100)

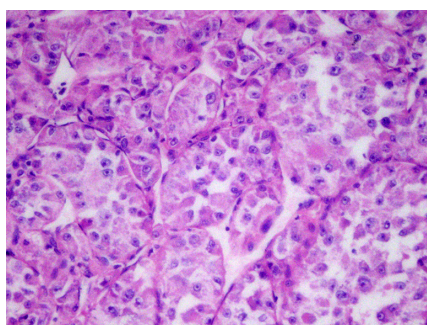


图4 肿瘤细胞圆形或多边形, 胞质丰富, 嗜酸性(HE, × 400)

Figure 4 Tumor cells are round or polygonal, abundant in cytoplasm and eosinophilic (HE, × 400)

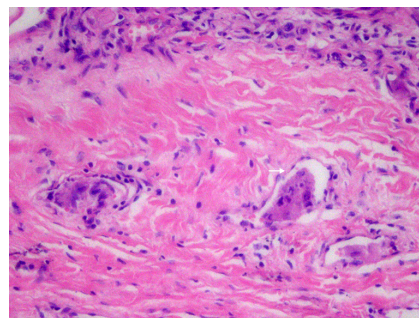


图5 箭头示血管内瘤栓形成(HE, × 200)

Figure 5 Arrow shows intravascular tumor emboli formation (HE, × 200)

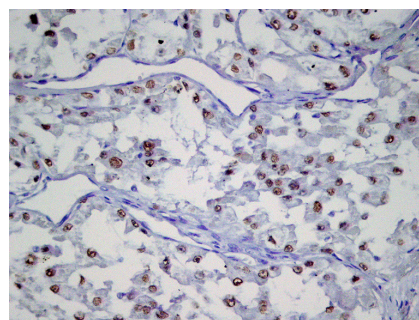


图6 肿瘤细胞表达TFE3(EnVision, × 400)

Figure 6 Tumor cells expressed TFE3 (EnVision, × 400)

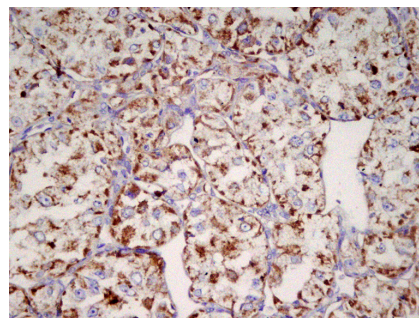


图7 肿瘤细胞细胞质表达MyoD1(EnVision, × 400)

Figure 7 Expression of MyoD1 in tumor cell cytoplasmic (EnVision, × 400)

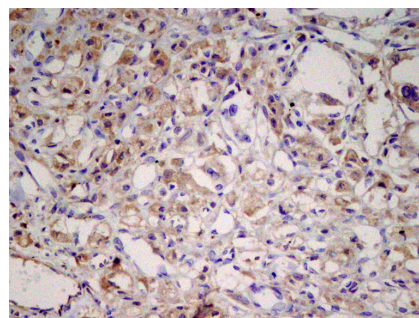


图8 肿瘤细胞细胞质表达Myogenin(EnVision, × 400)

Figure 8 Expression of Myogenin in tumor cell cytoplasmic (EnVision, × 400)

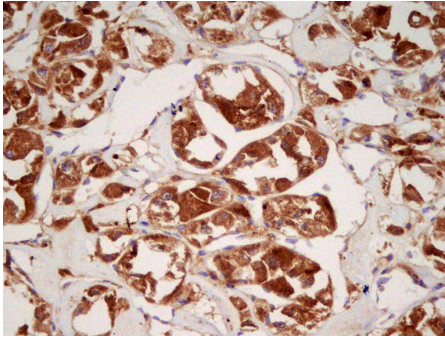


图9 肿瘤细胞细胞质表达NSE(EnVision, ×400)
Figure 9 Expression of NSE in tumor cell cytoplasmic (EnVision, ×400)

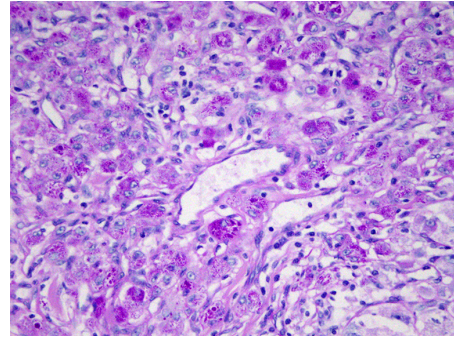


图12 肿瘤细胞细胞质伊红色细颗粒状, 其内见红染的棒状结晶(PAS, ×400)
Figure 12 Cytoplasm of the tumor cells is red and fine granular, and red dyed rod-like crystals are seen inside (PAS, ×400)

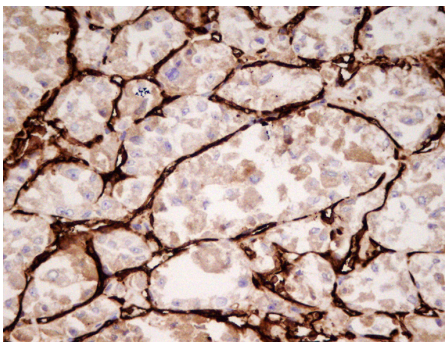


图10 CD31在肿瘤细胞内为阴性, 而在腺泡状结构间的血管壁为阳性(EnVision, ×400)
Figure 10 CD31 is negative in tumor cells and is positive in the wall of the blood between the alveolar structures (EnVision, ×400)

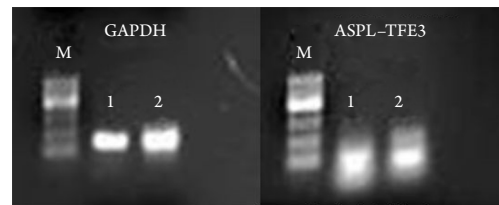


图13 肿瘤细胞表达ASPL-TFE3 1型融合基因(M: 标志物; 1/2: 本例样本)
Figure 13 Tumor cells expressed the ASPL-TFE3 type 1 fusion gene (M: maker; 1/2: the sample in this case)

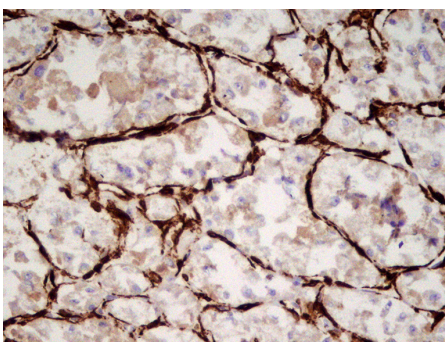


图11 SMA在肿瘤细胞内为阴性, 而在腺泡状结构间的血管壁为阳性(EnVision, ×400)
Figure 11 SMA is negative in tumor cells and is positive in the wall of the blood between the alveolar structures (EnVision, ×400)

2 讨论

临床上ASPS十分罕见, 占软组织肉瘤的0.5%~1.0%^[1-3], 好发于15~35岁, 女性多见。1952年Christopherson等首先命名了ASPS, 最常见的部位是下肢深部软组织, 也可见于口腔、眼眶、骨、乳腺、心脏、纵隔、胃、肝、腹膜后、膀胱、子宫和阴道等部位^[3-4]。儿童病例好发于头颈部, 尤其是舌和眼眶^[5]。ASPS为临床少见恶性肿瘤, 世界卫生组织(WHO)2013年公布的软组织与骨肿瘤分类将其定义为上皮样肿瘤, 组织病理学表现以相对一致、较大且富于嗜酸性和颗粒性胞质的上皮细胞样肿瘤组织呈实性巢状和/或腺泡状结构为特征^[6]。

ASPS病灶的肉眼观通常为黄白色、类球形或分叶状结节, 部分肿瘤呈“蘑菇征”^[7], 质地较软, 剖面多灰白, 部分病灶中央可见液化坏死^[3],

周边可见假性包膜^[4]。低倍镜下可见特征性的器官样结构, 肿瘤细胞被宽窄不等的纤维性间隔分割成多结节状, 肿瘤细胞呈腺泡状或片、巢状排列, 腺泡间血窦丰富^[8]。肺原发ASPS高倍镜下肿瘤细胞呈大而圆形, 大小和形状较一致, 具有明显上皮样, 胞质丰富、嗜酸性、细颗粒状; 可见空泡状核, 核仁明显, 核异型不明显, 核分裂象罕见^[3]。高倍镜下肿瘤细胞呈上皮样、圆形或多边形, 细胞界限清楚, 胞质丰富嗜酸性改变, 部分肿瘤细胞胞质透亮^[9]。本例患者肿瘤组织的CD31和Desmin染色: 肿瘤细胞团周围可见大量薄壁血管。细胞内含有大量细胞质, 细胞核呈圆形, 常有1个以上核仁, 细胞内常含有杆状或棒状结晶体, 经PAS染色后可显示^[10]。同时镜下多见血管内瘤栓, 可能为其较早出现转移的原因。在影像学上, ASPS患者的CT图像可见肿物外周对比增强以及中央液化坏死的现象^[2]。ASPS在MRI上具有一定的特征性表现: T1加权图像上为等信号或稍高信号影, T2加权图像上为高信号影、且呈不均匀强化, 绝大多数病灶内甚至病灶周边可呈现显著的流空效应, 且异构对比度增强明显^[2]。ASPS瘤体内存在大量迂曲的血管, 部分可伴动静脉瘘, 这些血管在MRI表现为流空信号。由于肿瘤组织内存在丰富血窦, 血流缓慢, 在T1WI上呈稍高信号, T2WI上呈高信号, 也可呈高低混杂信号, 由肿瘤组织内出血、坏死、瘢痕形成等因素所致^[11-12]。

临床上对于ASPS的来源一直存有较大争议, 因无可靠证据支持其他观点, 目前主流观点为肌源性来源^[13-14]。本研究免疫组织化学显示: 肌动蛋白(actin)、Desmin、波形蛋白(vimentin)等在ASPS中均有表达。Rosai等^[13]证实: ASPS组织中有MyoD1的表达, 而MyoD1是一种核磷蛋白, 具有控制肌源性细胞谱系发展的作用。Nakano等^[14]运用RT-PCR技术在ASPS组织中检测到MyoD1, $\alpha 2$ actin、原肌球蛋白的mRNA表达, 其中2例肌浆蛋白mRNA表达阳性, 并首次从分子水平提出ASPS为横纹肌源性肿瘤; 但其免疫组织化学显示肌相关标志在ASPS中的表达差异很大, Desmin的表达为40%。这可能与实验技术和方法有关。既往有大量研究利用免疫组织化学方法对ASPS进行研究, 但至今未发现有一致性的阳性结果。阳性主要表现为: MyoD1, Desmin, S-100, NSE, TFE3, Vin。阴性主要表现为: CK AE1/AE3, EMA, Syn, actin, MSA, SMA, CgA。长期以来, ASPS缺乏特异且敏感的免疫组织化学标志

物^[15]。正常肌肉组织内, MyoD1在细胞核中表达, 而ASPS则出现细胞质表达, 这可能与肿瘤的突变有关。本例中Desmin为阴性, 而MyoD1为细胞质阳性, 同样为肌源性标志物的Myogenin也显示细胞质阳性。另外NES阳性, 但此指标并不具有较大诊断价值。

ASPS中不平衡染色体易位der(17) t(X;17)(p11.2;q25)的发现, 为进一步探讨ASPS发生的分子机制提供线索, 这种不平衡易位导致Xp11.2上的TFE3转录因子基因与17q25上的ASPL(RCC17)基因发生融合, 形成ASPL/TFE-3融合基因, 作为一个异常的转录因子发挥作用, 从而引起肿瘤的发生^[16]。2001年, Ladanyi等^[17]运用RT-PCR技术首次在组织中检测到ASPL-TFE3融合基因mRNA的表达, 为进一步探讨ASPS的发生机制提供条件, 具有重要的诊断学意义^[18]。在本病例中检测到ASPL-TFE3融合基因的表达, 进而引起TFE3的过表达; 免疫组织同样得到证实, 该例患者肿瘤细胞强表达TFE3, 表明ASPL-TFE3融合基因在ASPS发病过程中起重要作用。提示ASPL-TFE3可以作为ASPS的一种合理的、特异的标志物。

此外, 腺泡状横纹肌肉瘤还需与其他肿瘤鉴别: 1)腺泡状横纹肌肉瘤, 属于小圆细胞恶性肿瘤。而ASPS形成腺泡状结构与腺泡状横纹肌肉瘤(alveolar rhabdomyosarcoma, ARMS)相似, 但ASPS的瘤细胞呈嗜伊红色的大多边形上皮样细胞, 腺泡之间为裂隙状或血窦样毛细血管网, 70%~80%的病例瘤细胞胞质内可见PAS阳性的针状或棒状结晶物, 血管内常见瘤栓^[19]。可呈腺泡状排列, 但腺泡之间为纤维血管间隔, 缺乏血窦, 瘤细胞表达Desmin, MyoD1, MSA等肌源性标志物。2)颗粒细胞瘤, 该肿瘤体积较小, 亦无明显腺泡状结构, 无毛细血管网的间质结构, 瘤细胞小, 无异型性, S100阳性, PAS染色阴性, 目前基本认为其来源于施万细胞。3)副节瘤, 腹膜后腹主动脉旁、肾门附近及下腔静脉旁最多见, 肿瘤组织由纤维条索分割成巢状, 肿瘤细胞由主细胞和支持细胞2种细胞, 主细胞表达CgA, Syn和神经内分泌等标志物, 支持细胞表达S100。4)原发性或转移性肾透明细胞癌, 癌细胞可排列成腺泡状, 血窦丰富, 但细胞核小, 细胞质透亮, 内无棒状结晶体。5)骨外尤文肉瘤, 其发病年龄更小, 常表现为肌肉内巨大肿块^[7]。ASPS亦需与恶性黑色素瘤、转移性腺癌等病进行鉴别。

ASPS病程较长, 为一种缓慢生长的肿瘤, 早

期症状极不明显, 因此有报道^[11]称ASPS为“无声肿瘤”, 但由于肿瘤的富血供性, 20%~25%的患者在就诊时就已发生转移。早期发现后, 行根治性手术是治疗的关键。治疗一般以手术为主, 辅以术后放化疗, 国内外关于靶向药物治疗的报道罕见。研究^[20]表明使用抑制血管生成的小分子靶向药物可能有一定疗效。本例患者术后行普通其他软组织肉瘤的化疗方案1个疗程后, 由于化疗后出现严重的不良反应而停药, 于2年后出现肺多发转移灶及髂骨转移病灶, 后行帕左帕尼(小分子抗血管生成靶向药)治疗后, 肿物明显缩小。目前病情趋于稳定, 但该肿瘤临床生物学行为难以确定, 需对患者进行长期随访。

综上所述, ASPS的预后并不理想, 主要归咎于肿瘤的局部复发和远处转移。患者的确诊年龄、原发灶的大小以及确诊时是否存在远处转移都是影响其预后的重要因素。临床鉴别需结合患者的临床病史、影像学检查、病理形态特点及免疫表型等来综合判定。目前临床上尚缺乏公认的早期确诊方法, 未来仍需进一步研究以完善这方面的工作。

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