·临床研究·

RNA 结合蛋白 PTBP1 与磷酸化-AXL 共表达在骨肉瘤中的临床意义

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摘　要：【目的】探索 RNA 结合蛋白 PTBP1 与 p-AXL 在骨肉瘤中的表达情况及其临床病理联系，并进一步探讨 PTBP1/p-AXL 共表达在骨肉瘤中的预后评估意义。【方法】应用 GEO 和 Target 数据库分析 PTBP1 与 AXL 在骨肉瘤和正常组织中的表达差异及 PTBP1 的预后价值。收集 2016 年 3 月至 2020 年 10 月中山大学第一附属医院 76 例骨肉瘤和 37 例非恶性骨组织(骨痂、骨纤维结构不良或骨样骨瘤)及其病例信息，应用免疫组化法检测 PTBP1 和 p-AXL 蛋白的表达情况并行统计学分析。【结果】GEO 数据库分析结果显示 PTBP1 和 AXL 在骨肉瘤组织的表达具有高于正常组织的趋势，但尚未达到统计学意义；Target 数据分析结果显示 PTBP1 高表达组骨肉瘤患者总生存期(OS)与无进展生存期(PFS)均短于低表达组，但差异尚未达统计学意义(P=0.064；P=0.134)。免疫组化结果显示 PTBP1 和 p-AXL 蛋白的表达在骨肉瘤组织中显著高于非恶性骨组织；p-AXL 阳性表达率与肺转移相关(P=0.025)；Kaplan-Meier 分析发现肺转移、复发、PTBP1 表达及 PTBP1/p-AXL 共表达等因素与骨肉瘤患者不良总生存期相关；且多变量 Cox 回归分析显示肺转移(P<0.000 1)、PTBP1 表达阳性(P=0.041)是骨肉瘤患者总生存期(OS)独立危险因素；PTBP1/p-AXL 共表达患者的 OS(P=0.017)和 PFS(P=0.043)均低于非 PTBP1/p-AXL 共表达患者。【结论】PTBP1、p-AXL 在骨肉瘤中高表达，PTBP1 与 p-AXL 共表达与患者预后差相关，且 PTBP1 可作为骨肉瘤患者独立预后指标。

关键词：骨肉瘤；多聚嘧啶区结合蛋白 1；受体酪氨酸激酶；预后
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Clinical Significance of co-expression of RNA Binding Protein PTBP1 and p-AXL in Osteosarcoma

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Abstract:【Objective】To explore the co-expression of PTBP1 and p-AXL in osteosarcoma and its clinicopathological significance for prognosis evaluation.【Methods】The expression of PTBP1 and AXL and their prognostic value in osteosarcoma were analyzed by GEO and Target data. Paraffin biopsy specimens and clinical information from 76 cases of osteosarcoma and 37 cases of non-malignant bone tissue (callus, osteofibrous dysplasia and osteoid ostema) were obtained from the First Affiliated Hospital of Sun Yat-sen University from March 2016 to October 2020. The expressions of PTBP1 and p-AXL proteins in osteosarcoma were detected by immunohistochemistry.【Results】GEO database showed that the expression

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levels of PTBP and AXL in osteosarcoma tumor group were higher than those in normal tissues, but did not reach statistical significance. Target database showed that the high expression of PTBP1 had shorter Overall survival(OS) and Progression-free survival(PFS) than low PTBP1 expression, but did not reach statistical significance (P=0.064; P=0.134). Immunohistochemical staining included 76 cases of osteosarcoma and 37 cases of non-malignant bone tissue. The expression rate of PTBP1 and p-AXL protein in osteosarcoma tissues was higher than that in non-malignant bone tissue. The expression of p-AXL is correlated with lung metastasis (P=0.025). Kaplan-Meier analysis showed that lung metastasis, recurrence, PTBP1 expression, co-expression of PTBP1/p-AXL influence the prognosis of patients in OS. Multivariate Cox regression analysis showed that lung metastasis (P<0.000 1) and positive expression of PTBP1 (P=0.041) were independent risk factors for osteosarcoma patients in OS. Co-expression of PTBP1 and p-AXL had shorter OS (P=0.017) and PFS (P=0.043) than non-coexpression osteosarcoma patients.【Conclusions】PTBP1 and p-AXL were highly expressed in osteosarcoma tissues. The co-expression of PTBP1 and p-AXL was associated with poor prognosis of patients, and PTBP1 could be used as an independent prognostic indicator of patients with osteosarcoma.

Key words: osteosarcoma; PTBP1; p-AXL; prognosis

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骨肉瘤(osteosarcoma, OS)最常见的原发成骨性恶性肿瘤,具有两个发病高峰,第一个是14-18岁的青少年,第二个为60岁以上的老年人,其侵袭性高,尤其是远处转移患者,预后极差,社会危害性大[1-2]。尽管多学科综合治疗取得一定进展,但术后复发、转移率高仍是导致骨肉瘤患者总生存期短、生活质量低的主要原因[3-5]。因此,深入研究骨肉瘤的发生发展机制,将有助于发现骨肉瘤患者预后的有效生物标志物以及开发靶向疗法。多聚嘧啶区结合蛋白1(polypyrimidine tract binding protein 1, PTBP1)是一种RNA结合蛋白,属于普遍表达的异质核糖核蛋白(hnRNPs)亚家族成员,其基因位于人类19p13.3染色体上[6]。PTBP1在恶性肿瘤中发挥的作用已成为近年来的研究热点,研究结果显示在肾细胞癌和乳腺癌中PTBP1通过不同的途径和分子机制发挥调节细胞增殖、迁移和侵袭的作用,从而促进肿瘤的发生发展[7-9]。最近有研究报道,LncRNA HOTTIP通过与PTBP1相互作用提高KHSRP蛋白表达,从而促进骨肉瘤细胞增殖、侵袭和迁移[10]。虽然PTBP1在多种疾病中已有广泛研究,但是PTBP1影响骨肉瘤发生发展的相关机制尚未明确;其作为生物标志物和治疗靶点仍缺乏理论基础。受体酪氨酸激酶(receptor tyrosine kinase, AXL)是TAM(Tyro3, AXL, Mer)家族成员[11],其特异性配体Gas6能够与AXL的胞膜外免疫球蛋白样结构域结合,随后Gas6/AXL磷酸化触发多种信号通路从而调控细胞增殖、侵袭和转移等过程[12-13]。本课题组前期研究结果表明骨肉瘤细胞株中应用人重组Gas6刺激活化AXL后,瘤细胞的体外迁移和侵袭能力显著增强[14]。本研究拟通过检测骨肉瘤组织中PTBP1及p-AXL蛋白的表达水平,探索PTBP1及p-AXL在骨肉瘤中的表达情况及其临床意义,并探讨PTBP1/p-AXL蛋白共表达的临床病理意义。

1　材料与方法

1.1　选择入组病例

收集2016年3月至2020年10月中山大学第一附属医院76例骨肉瘤和37例非恶性骨组织(骨痂组织、骨纤维结构不良或骨样骨瘤)的石蜡活检标本(FFPE)。入排标准:①患者活检前未接受化疗;②具有完整的临床、影像学、病理及随访资料。本研究已获得中山大学(中国广州)第一附属医院伦理委员会批准免知情同意。

1.2　GEO数据分析PTBP1和AXL在正常组织和骨肉瘤组织中表达情况

下载基因表达汇编(Gene Expression Omnibus, GEO)数据库中的GSE99671及相应的临床信息,包含18个正常样本和18个肿瘤样本。通过R语言(4.2.2版本)中的R包"ggpubr"进行差异箱线图绘制。

1.3　Target数据分析PTBP1对骨肉瘤患者预后影响

从Target数据库(https://ocg.cancer.gov/pro-

grams/target)下载骨肉瘤的表达谱数据和相应的临床信息。删除缺少生存信息的病例,最终获得生存状态样本95例。通过R语言中的"survival survminer" R包进行连续变量生存曲线绘制。

1.4 免疫组织化学染色

采用PV6000免疫组化试剂盒(北京中山金桥生物科技有限公司)对PTBP1和p-AXL进行免疫组化检测。所有标本在室温下用10%的福尔马林固定12~24 h。石蜡包埋标本连续切片(厚4 μm),60 ℃孵育2 h,脱蜡脱水,切片在柠檬酸缓冲液(pH值6.0)或EDTA(pH值9.0)中高压修复2 min/30 s,然后在自来水下冷却至室温。3%双氧水室温孵育30 min,阻断内源性过氧化物酶活性。普通山羊血清(Jiangsu Cowin Biotech)作为阻断剂,减少组织与抗体的非特异性结合。抗p-AXL抗体(AF8523,1∶200稀释,Affinity,USA)和PTBP1(67462-1-Ig,1∶400稀释,Proteintech,China),4 ℃孵育14~16 h。次日室温静置30 min,酶标记山羊抗鼠/兔IgG聚合物(北京中山金桥生物科技有限公司)室温孵育30 min。DAB(Jiangsu Cowin Biotech)显色1~3 min,苏木精复染1 min,冲洗,过梯度乙醇和二甲苯,中性树脂封片。

所有免疫组化结果,由两名高年资病理医师进行双盲判读,判读不一致时讨论后统一意见。PTBP1在肿瘤细胞的胞核中染色为阳性表达,p-AXL在肿瘤细胞的胞核和/或胞浆染色为阳性表达。阳性肿瘤细胞占所有肿瘤细胞的百分比为,0∶0%;1∶≤25%;2∶26%~50%;3∶51%~75%;4∶76%~100%;染色强度分为,0∶无着色;1+∶淡黄色;2+∶棕黄色;3+∶棕褐色。两项评分相乘为最终得分(阳性百分比×强度)。PTBP1和p-AXL免疫组化评分≥2为阳性表达;0分或1分为阴性表达。

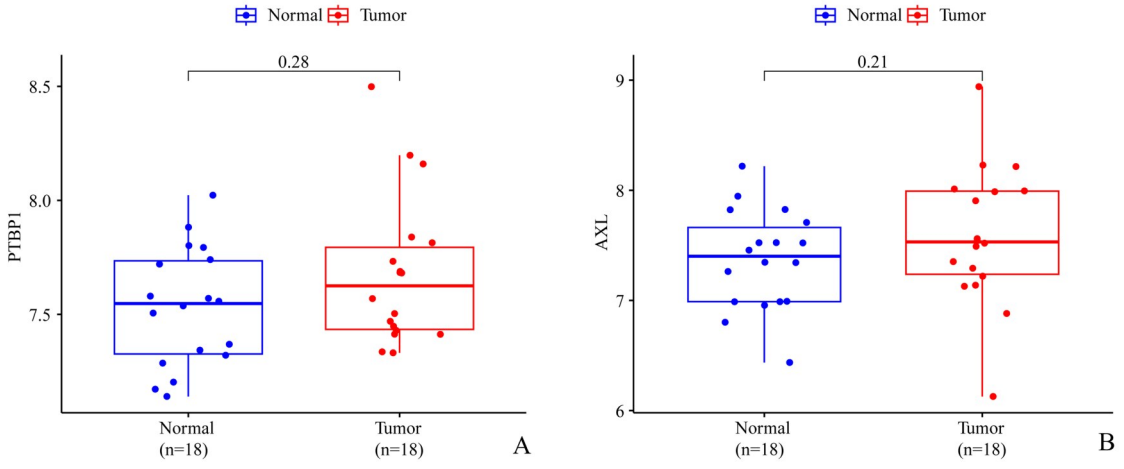
1.5 统计学分析

应用SPSS25.0及Graphpad Prism 8统计软件进行统计学分析。PTBP1和p-AXL与临床病理特征相关性分析采用卡方检验;无进展生存期(PFS)定义为病理明确诊断骨肉瘤至疾病进展、复发或死亡的时间;总生存期(OS)定义为病理明确诊断骨肉瘤至因肿瘤相关死亡或最后一次随访的时间;用Kaplan-Meier法绘制生存曲线,采用log-rank检验比较生存曲线。使用Cox比例风险模型进行多因素分析,以$P<0.05$认为差异具有统计学意义。

2 结果

2.1 GEO数据库比较正常组织与骨肉瘤中PTBP1和AXL mRNA水平

GEO数据库分析18例正常组织和18例骨肉瘤组织,结果显示PTBP1 mRNA和AXL mRNA在骨肉瘤组织中的表达量具有高于正常组织的趋势(图1),但是差异无统计学意义($P=0.28$;$P=0.21$)。



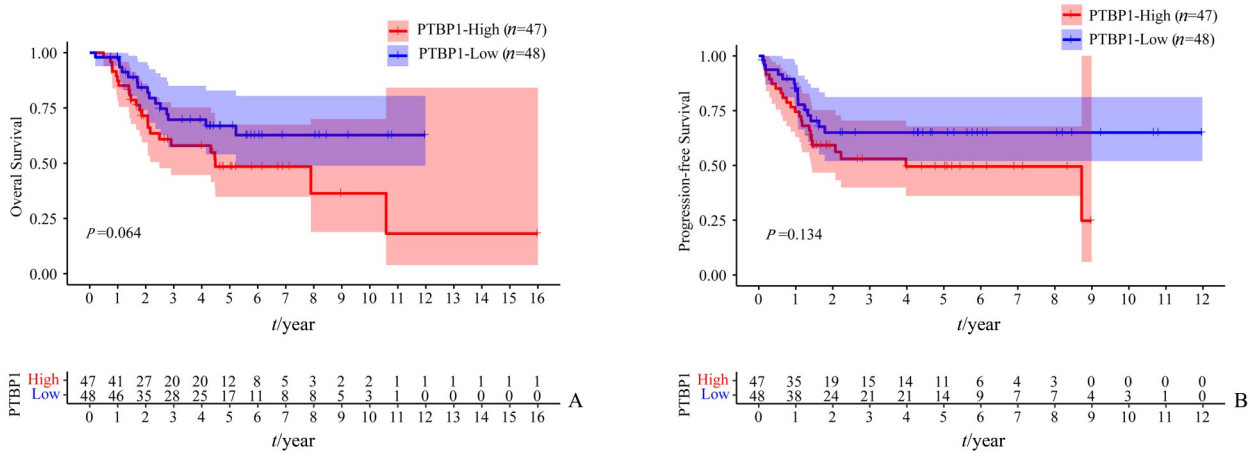
A: Bioinformatics analysis of the expression of PTBP1 in osteosarcoma tissues (n=18) was higher than non-tumor tissues (n=18) based on GEO data, but the difference had not statistically significance (P=0.28). B: Bioinformatics analysis of the expression of AXL in osteosarcoma tissues (n=18) was higher than non-tumor tissues (n=18) based on GEO data, but the difference was not statistically significant (P=0.21).

图1 GEO数据库中PTBP1和AXL在正常组织和骨肉瘤组织的表达水平对比

Fig. 1 Comparison of expression levels of PTBP1 and AXL in normal tissues and osteosarcoma tissues in GEO database

2.2　Target数据库骨肉瘤组织PTBP1 mRNA水平与预后相关性分析

从Target数据库下载的95例骨肉瘤患者预后信息，以中位数（median=6.851，range 5.758～8.273）为界值，分为PTBP1 mRNA高表达组（n=47）与低表达组（n=48），使用R包“survival survminer”进行Kaplan-Meier单因素分析，结果显示PTBP1 mRNA高表达组患者OS与PFS均短于低表达组（图2），但差异均无统计学意义（P=0.064；P=0.134）。



A：Bioinformatics analysis of Target data showed that high level of PTBP1 (n=47) predicted the trend of poor prognosis of OS than low level of PTBP1(n=48), but the difference was not statistically significant (P=0.064). B: Bioinformatics analysis of Target data showed that high level of PTBP1 predicted the trend of poor prognosis of PFS, but the difference was not statistically significant (P=0.134). OS: overall survival; PFS: progression-free survival.

图2　通过Target数据库中分析PTBP1mRNA水平对骨肉瘤患者OS与PFS的影响

Fig. 2　Analysis the prognostic effect of PTBP1 mRNA levels on OS and PFS in patients with osteosarcoma through Target database

2.3　本组骨肉瘤患者临床病理特征

本研究中纳入的76例骨肉瘤患者包括男性44名，女性32名；年龄范围4～62岁，平均年龄17.9岁，中位数年龄15岁；其中肺转移患者33名，复发患者12名。患者临床病理资料如表1所示。

2.4　PTBP1蛋白在骨肉瘤组织中的表达

免疫组化染色结果显示PTBP1在细胞核中表达（图3）。对所有病例进行PTBP1免疫组化评分，发现47例（61.8%）骨肉瘤组织（OS）PTBP1表达阳性，即免疫组化评分≥2；29例（38.2%）PTBP1免疫组化表达阴性。37例非恶性骨组织（Non-OS）中13例（35.1%）PTBP1表达阳性，24例（64.9%）PTBP1蛋白阴性表达。相关性分析显示骨肉瘤组织中PTBP1阳性表达率高于非恶性骨组织（Non-OS），且差异具有统计学意义（χ^2=7.127，P=0.008；表2）。

2.5　p-AXL蛋白在骨肉瘤组织中的表达

免疫组化染色结果显示p-AXL在细胞核和细胞质中均有表达（图4）。对所有病例进行了p-AXL免疫组化评分，60例（78.9%）OS组织p-AXL阳性表达，即免疫组化评分≥2；16例（21.1%）骨肉瘤组织（OS）p-AXL阴性表达。37例非恶性骨组织（Non-OS）中20例（54.1%）p-AXL阳性表达，17例（45.9%）p-AXL免疫组化评分阴性。相关性分析显示骨肉瘤组织中p-AXL阳性表达率高于非恶性骨组织，差异同样具有统计学意义（χ^2=7.459，P=0.006；表2）。

2.6　PTBP1及p-AXL蛋白表达与骨肉瘤患者临床病理特征的相关性研究

经χ^2检验分析发现，本组病例p-AXL阳性表达与肺转移率相关（P=0.025）。但p-AXL的表达情况与性别、年龄、肿瘤直径、复发与否、生存状况等临床病理特征中差异均无统计学意义。PTBP1表达情况与上述临床病理特征均差异无统计学意义（表1）。

2.7　PTBP1及p-AXL蛋白表达及各临床病理参数对患者预后影响分析

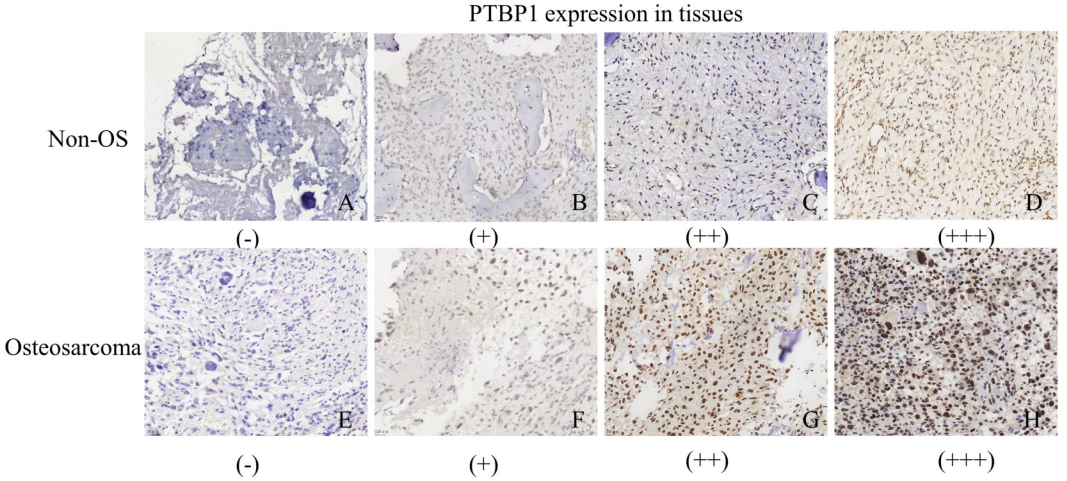
为评估不同参数对骨肉瘤患者的预后价值，对可能影响骨肉瘤患者生存时间的参数进行Kaplan-

表1 PTBP1与p-AXL与骨肉瘤临床病理特征的关系

Table 1 The relationship between the PTBP1 and p-AXL and clinic-pathological features

Clinicpathological Characterstics		PTBP1				p-AXL			
		Total	+	-	*P*	Total	+	-	*P*
Gender									
	Male	44	29	15	0.392	44	35	9	0.881
	Female	32	18	14		32	25	7	
Age									
	<18	49	32	17	0.402	49	38	11	0.688
	≥18	27	15	12		27	22	5	
Tumor diameter (cm)	<8	30	19	11	0.829	30	22	8	0.332
	≥8	46	28	18		46	38	8	
Pulmonary Metastasis									
	YES	33	22	11	0.448	33	30	3	0.025[1)]
	NO	43	25	18		43	30	13	
Recurrence									
	YES	12	8	4	0.959	12	10	2	0.984
	NO	64	39	25		64	50	14	
Survival status									
	Alive	43	23	20	0.087	43	32	11	0.269
	Dead	33	24	9		33	28	5	

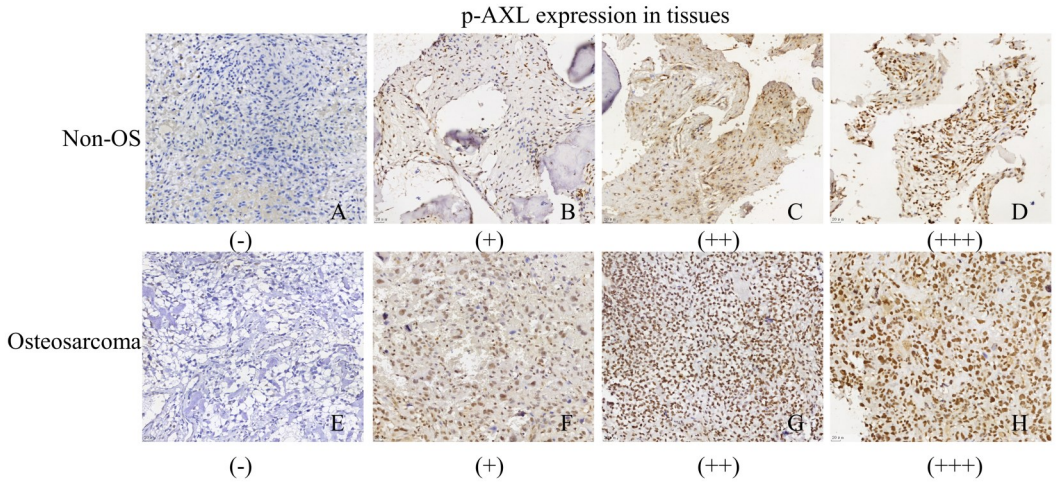
1) χ^2 =5.021, P = 0.025.



A, E: negative (－, ×400); B,F: weak expression (+, ×400); C, G: moderate expression (2+, ×400); D, H: strong expression (3+, ×400); A-D: non-malignant bone tissue; E-H: osteosarcoma.

图3 PTBP1在非恶性骨组织和骨肉瘤组织中的表达情况

Fig. 3 The expression of PTBP1 in non-malignant bone tissues and osteosarcoma tissues



A, E: negative (– , ×400); B, F: weak expression (+, ×400); C, G: moderate expression (2+, ×400); D, H: strong expression (3+,×400); A–D: non-malignant bone tissue; E–H: osteosarcoma.

图4　p-AXL在非恶性骨组织和骨肉瘤组织中的表达情况

Fig. 4　The expression of p-AXL in non-malignant bone tissues and osteosarcoma tissues

表2　正常组织与骨肉瘤组织中PTBP1和p-AXL表达相关性分析

Table 2　The expression of PTBP1 and p-AXL in normal tissues and osteosarcoma tissues

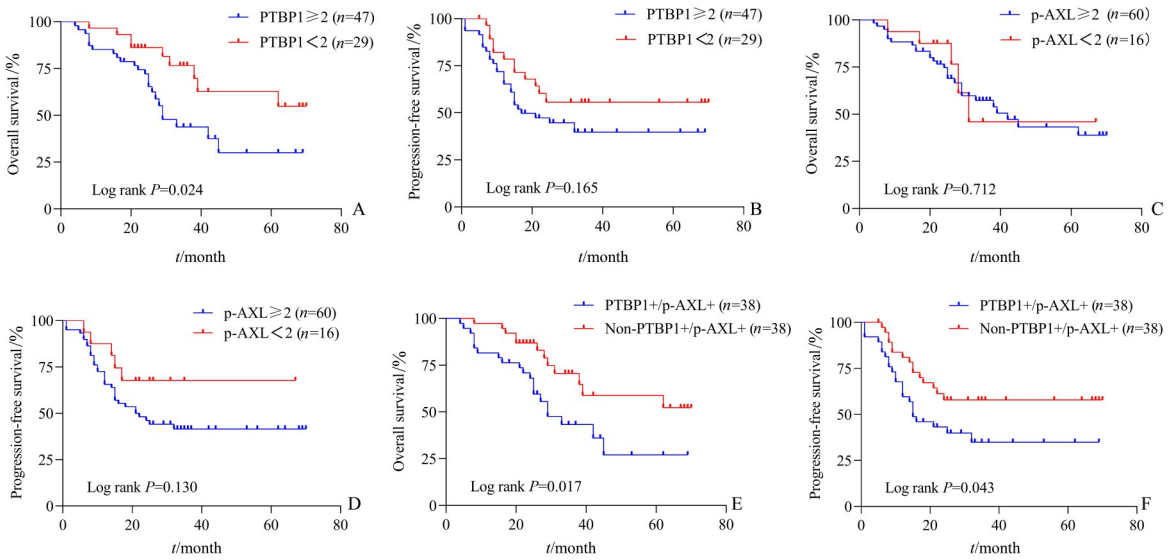
Expression of protein	Non-malignant bone tissue	Osteosarcoma	χ^2	P
PTBP1			7.127	0.008[1)]
Negative (-)	24	29		
Positive (+)	13	47		
p-AXL			7.459	0.006[2)]
Negative (-)	17	16		
Positive (+)	20	60		

1) ϕ=0.251; 2) ϕ=0.257

Meier法单因素分析，发现骨肉瘤肺转移患者的平均生存期（29.3月），肺转移患者中位生存期为25.0月（21.4～28.6月），短于未出现肺转移患者的平均生存期（57.7月，χ^2=20.921，P<0.000 1）；骨肉瘤复发患者的平均生存期（22.1月），中位生存期为21.0月（13.3～28.7月），短于未复发患者的平均生存期（47.8月，χ^2=12.129，P<0.000 1）；PTBP1阴性组的平均生存期（53.7月）相对于阳性组（38.2月）总生存期更长（χ^2=5.074，P=0.024；表3、图5），阳性组中位生存期为29.0月（21.7～36.4月）。由于未出现肺转移、未复发及PTBP1阴性表达组患者在此次随访时间内死亡人数未达到50%，均尚未出现中位生存期。PTBP1阳性组无进展生存期也短于阴性组，但差异无统计学意义（χ^2=1.925，P=0.165）；而p-AXL表达情况与骨肉瘤患者OS和PFS均差异无统计学意义；PTBP1和p-AXL共表达的骨肉瘤患者OS与PFS均短于非共表达骨肉瘤患者，且差异具有统计学意义（OS，χ^2=5.748，P=0.017；PFS，χ^2=4.080，P=0.043；图5）。

2.8　骨肉瘤患者独立预后因素分析

为进一步分析骨肉瘤患者独立预后因素，对有无肺转移、有无复发、PTBP1表达情况进行Cox多因素回归分析（backward：LR）结果显示肺转移[HR=4.973，95%CI（2.284～10.828），P<0.000 1]和PTBP1阳性表达[HR=2.281，95%CI（1.036，5.024），P=0.041]是骨肉瘤患者OS独立危险因素（表4）；而仅肺转移[HR=17.867，95%CI（6.568，48.604），P<0.000 1]是骨肉瘤患者PFS独立危险因素（表5）。



A, B Kaplan-Meier analysis showed that patients with PTBP1 positive expression had poor prognosis for OS (P=0.024) and PFS (P=0.165), the latter difference had not statistically significance. C, D Kaplan-Meier analysis showed that patients with p-AXL positive expression had no statistical significance in OS (P=0.712) and PFS (P=0.130). E, F Kaplan-Meier analysis showed that patients with positive co-expression of PTBP1 and p-AXL had poor prognosis for OS (P=0.017) and PFS (P=0.043) in osteosarcoma.

图5 生存曲线分析

Fig. 5 Survival curves of the patients

3 讨 论

自从新辅助化疗联合手术切除成为骨肉瘤主要治疗方式后,局限性骨肉瘤患者5年无进展生存率显著提高至约70%,但转移性和复发性骨肉瘤患者总生存率仍低于20%[15]。目前,骨肉瘤侵袭转移的相关机制尚未明确。因此,对骨肉瘤进行更多的临床病理研究有助于了解骨肉瘤的发病机制,为评估临床预后提供更多的理论依据。

PTBP1是一种多功能的RNA结合蛋白,其作用包括选择性剪接,介导翻译起始和维持mRNA稳定性[16],近年来,PTBP1逐渐成为骨肉瘤领域研究热点。Zhang等[17]研究发现与正常组织对比,PTBP1在骨肉瘤中高表达,同时在骨肉瘤组织化疗耐药组患者PTBP1表达高于化疗敏感组,且预后不良。Li等[18]人研究同样发现与瘤旁组织对比,PTBP1在骨肉瘤中高表达,其认为CircFam120b海绵mi-R-1205上调PTBP1的表达而促进骨肉瘤进展。目前仅有1篇文献报道PTBP1蛋白在骨肉瘤表达高于瘤旁组织,但其样本量仅30例,且缺少预后信息及临床特征相关性分析[19]。另有研究表明PTBP1在肿瘤细胞生长和维持、肿瘤转移、有氧糖酵解及肿瘤耐药中发挥重要作用[20-22]。本研究通过生物信息学分析,发现在骨肉瘤中PTBP1 mRNA在骨肉瘤组织的表达高于正常组织,且PTBP1高表达患者预后不良,但尚未达到统计学意义,这可能与数据库样本中缺少亚洲人群数据、可分析样本量少及肿瘤异质性等因素有关。然而,本研究通过免疫组化法检测PTBP1在76例骨肉瘤组织中的蛋白表达水平,却发现骨肉瘤中PTBP1蛋白水平显著性高于非恶性骨组织(Non-OS);PTBP1阳性表达患者的总生存期亦显著性低于PTBP1阴性组,其无进展生存期也具有相对较差的趋势。我们又进行了Cox多因素回归分析,发现PTBP1是骨肉瘤患者总生存期独立预后因素,提示PTBP1有望成为评估骨肉瘤患者预后的新指标。

TAM家族受体酪氨酸激酶(RTK)由TYRO3、AXL和MERTK组成,它们由配体GAS6和蛋白S激活[23]。目前多数研究认为AXL在癌细胞存活、转移和耐药中发挥关键作用,如AXL表达与乳腺癌、卵巢癌等多种肿瘤的转移风险增加和生存率低有关[24-26]。文献报道约50%骨肉瘤患者在首诊或治疗期间可出现肺转移,肺转移者5年生存率由82.0%降至38.3%[27],在本组病例中,33例(43.4%)骨肉瘤患者出现肺转移,死亡人数24名,平均生存期29.3月,5年生存率27.3%,患者资料与文献报道

表3　单变量分析PTBP1和p-AXL及临床病理参数与76例骨肉瘤患者总生存期相关性

Table 3　Analysis of PTBP1 and p-AXL protein expression and clinicopathological parameters in the overall survival of 76 patients with osteosarcoma

Variables		*n*	Mean survival/months	*P*
Gender				
	Male	44	45.4	0.644
	Female	32	42.3	
Age				
	＜18	49	42.8	0.711
	≥18	27	46.8	
Tumor diameter/cm				
	＜8	30	41.2	0.591
	≥8	46	45.7	
Pulmonary Metastasis				
	YES	33	29.3	＜0.000[1)]
	NO	43	57.7	
Recurrence				
	YES	12	22.1	＜0.000[2)]
	NO	64	47.8	
PTBP1				
	Positive	47	38.2	0.024[3)]
	Negative	29	53.7	
p-AXL				
	Positive	60	44.0	0.712
	Negative	16	44.2	
Co-expression	PTBP1+/p-AXL+	38	36.6	0.017[4)]
	Non-PTBP1+/p-AXL+	38	52.0	

1）χ^2 =20.921 P<0.000；2）χ^2 =12.129 P<0.000；3）χ^2 =5.074 P=0.024；4）χ^2 =5.748 P=0.017.

表4　76例骨肉瘤患者OS的多变量Cox回归结果(1)

Table 4　Multivariate cox analysis of OS in 76 patients with osteosarcoma（part 1）

Variable	*b*	S_b	Wald χ^2	*P*	HR	HR 95%CI
Pulmonary Metastasis	1.604	0.397	16.320	0.000	4.973	(2.284,10.828)
PTBP1+	0.825	0.403	4.190	0.041	2.281	(1.036,5.024)

表5　76例骨肉瘤患者PFS的多变量Cox回归结果(2)

Table 5　Multivariate cox analysis of PFS in 76 patients with osteosarcoma（part 2）

b	S_b	Wald χ^2	*P*	HR	HR 95%CI
2.883	0.511	31.879	0.000	17.867	(6.568,48.604)
0.717	0.372	3.712	0.054	2.048	(0.988,4.246)

一致。本课题组前期研究结果表明骨肉瘤细胞AXL活化后,瘤细胞的迁移和侵袭能力显著增强[14];且AXL高表达骨肉瘤细胞族群通过释放含Linc00852的外泌体,促进低表达AXL的骨肉瘤细胞生长、侵袭和转移[28]。本研究通过生信分析发现AXL mRNA在骨肉瘤组织的表达具有高于正常组织的趋势;免疫组化检测证实p-AXL蛋白在骨肉瘤组织中高表达,且p-AXL阳性表达其肺转移率显著性升高(P=0.025);p-AXL蛋白阳性表达组的无进展生存期低于阴性表达组,其差异尚未达统计学意义,这可能受限于样本量有限,尚需积累更多病例进一步验证。根据前期研究结果及本实验结果推测AXL在骨肉瘤发生发展过程中起关键作用是其活化形式或存在转录后调控机制,但p-AXL与肺转移之间的分子调控机制有待进一步研究。

最近研究发现在肺肿瘤中AXL转录率无显著差异,但PTBP1通过降低AXL mRNA稳定性来调控AXL的表达,从而抑制肿瘤发生发展[29]。Shen等[30]研究发现PTBP1作为一种重要的剪接调节剂,可选择性剪接AXL pre-mRNA,增加AXL-S蛋白表达,从而促进肝癌细胞的侵袭、转移。上述研究提示在不同肿瘤中PTBP1与AXL之间存在某些调控机制,为进一步探究PTBP1/p-AXL共表达对骨肉瘤患者预后影响,我们对患者进行Kaplan-Meier分析单因素分析,发现该类患者的OS(P=0.017)和PFS(P=0.043)均低于非PTBP1/p-AXL共表达患者。因此PTBP1与AXL在骨肉瘤中可能存在某种精细的分子调控机制,尚需进一步深入研究。

综上所述,PTBP1及p-AXL蛋白在骨肉瘤组织中高表达;PTBP1是骨肉瘤患者总生存期独立预后因素,p-AXL蛋白表达与骨肉瘤肺转移具有相关性,PTBP1/p-AXL共表达对骨肉瘤患者预后具有一定提示意义,提示PTBP1和p-AXL共表达可能成为骨肉瘤患者预后评估及靶向性治疗的潜在靶标。

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