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非小细胞肺癌 Survivin，Skp2 和 XIAP mRNA 的表达及其临床意义

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摘要 目的：探讨非小细胞肺癌（NSCLC）生存素（Survivin）、S 期激酶相关蛋白 2（Skp2）和 X 连锁凋亡抑制蛋白（XIAP）mRNA 的表达及其临床意义。**方法：**收集 2014 年 4 月到 2017 年 5 月期间我院 182 例 NSCLC 患者在手术中切除的病理组织作为研究组，另收集每例患者癌变组织旁 5cm 以外的癌旁组织作为对照组。比较两组的 Survivin、Skp2 和 XIAP mRNA 阳性率，并分析 Survivin、Skp2 和 XIAP mRNA 表达与临床病理特征的关系。**结果：**研究组的 Survivin、Skp2 和 XIAP mRNA 阳性率显著高于对照组，差异有统计学意义（P<0.05）。Survivin mRNA 的阳性表达与年龄、性别、组织类型、分化程度、临床分期、淋巴结转移、吸烟史无关（P>0.05）。Skp2 mRNA 的阳性表达与年龄、性别、组织类型、分化程度、淋巴结转移、吸烟史无关（P>0.05），与临床分期相关（P<0.05）。XIAP mRNA 的阳性表达与年龄、性别、组织类型、分化程度、吸烟史无关（P>0.05），与临床分期、淋巴结转移相关（P<0.05）。**结论：**Survivin、Skp2 和 XIAP mRNA 在 NSCLC 患者的病理组织中呈高表达，Skp2 mRNA 的阳性表达与临床分期有关，XIAP mRNA 的阳性表达与临床分期、淋巴结转移有关。

关键词：非小细胞肺癌；生存素；细胞 S 期激酶相关蛋白 2；X 连锁凋亡抑制蛋白；临床意义

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Expression and Clinical Significance of Survivin, Skp2 and XIAP mRNA in Non-Small Cell Lung Cancer

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ABSTRACT Objective: To investigate the expression and clinical significance of survivin(Survivin), S-phase kinase associated protein 2(Skp2)and X-linked inhibitor of apoptosis protein (XIAP) mRNA in non-small cell lung cancer（NSCLC）. **Methods:** The pathological tissues of 182 patients with NSCLC, who underwent surgical resection in Third People's Hospital of Yunnan Province during April 2014 to May 2017, were collected as study group; in addition, the paracancerous tissues outside 5cm beside the cancerous tissue of each patient were collected as control group. The positive rates of Survivin, Skp2 and XIAP mRNA in the two groups were compared, and the relationship between the expressions of Survivin, Skp2 and XIAP and clinicopathological features of mRNA was analyzed. **Results:** The positive rates of Survivin, Skp2 and XIAP mRNA in the study group were significantly higher than those in the control group, and the difference was statistically significant (P<0.05). The positive expression of Survivin mRNA was not related to age, sex, tissue type, differentiation degree, clinical stage, lymph node metastasis and smoking history (P>0.05). The positive expression of Skp2 mRNA was not related to age, sex, tissue type, differentiation degree, lymph node metastasis and smoking history (P>0.05), but related to clinical stage (P<0.05). The positive expression of XIAP mRNA was not related to age, sex, tissue type, differentiation degree, smoking history (P>0.05), but related to clinical stage and lymph node metastasis (P<0.05). **Conclusion:** Survivin, Skp2 and XIAP mRNA are highly expressed in the pathological tissues of patients with NSCLC. The positive expression of Skp2 mRNA is related to the clinical stage, and the positive expression of XIAP mRNA is related to the clinical stage and lymph node metastasis.

Key words: Non-small cell lung cancer; Survivin; Skp2; XIAP; Clinical significance

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前言

肺癌是一种肺实质部的癌症，严重危害人类的健康和生命，发病原因多与吸烟、生活环境等密切相关[1]。近年来肺癌的发病率逐年上升并占据全世界及我国恶性肿瘤发病率、死亡率首位，五年生存率低于 20%[2]。肺癌早期症状较轻甚至无症状，随着病情的发展才逐渐出现咳嗽、痰中带血、咯血、胸痛、消瘦等症状，但上述症状均缺乏特异性，往往难以引起患者重视，因此大多数患者在确诊为肺癌时已经到了晚期，错过了手术切除的最佳时机，对治疗和预后均有严重的影响，所以针对肺癌的早期诊断已成为现今科研人员研究的热点问题之一[3,4]。非小细胞肺癌（Non-Small Cell Lung Cancer，NSCLC）是肺癌最为常见

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的一种类型，约占肺癌患者总数的85%左右，因此临床上多从NSCLC切入对肺癌进行研究。生存素（Survivin）隶属凋亡抑制蛋白家族，其在正常的组织细胞中呈现低表达或不表达，但在肺癌、乳腺癌、肝癌、前列腺癌等恶性肿瘤中均异常表达，其表达程度与肿瘤的发生、发展密切相关[5-7]。S期激酶相关蛋白2（S-phase kinase associated protein 2，Skp2），是属于泛素化-蛋白酶系统的细胞周期蛋白，参与介导细胞增殖和细胞周期的进程，能够影响多种恶性肿瘤的转移、浸润[8]。X连锁凋亡抑制蛋白（XIAP）是凋亡抑制蛋白家族的成员，能够抑制细胞凋亡[9]。本研究旨在探讨NSCLC中Survivin、Skp2和XIAP mRNA的表达及其临床意义，以进一步了解肺癌与Survivin、Skp2和XIAP的内在联系，现将研究内容整理如下。

1 资料与方法

1.1 一般资料

收集2014年4月到2017年5月期间我院182例NSCLC患者在手术中切除的病理组织作为研究组，纳入标准：① 均经病理检测诊断为NSCLC；② 患者对本次研究知情同意。排除标准：① 术前接受过放化疗治疗；② 临床资料不完整。182例NSCLC患者中，男101例，女81例，年龄36-84岁，平均年龄（67.7± 8.6）岁；病理组织类型：腺癌96例，鳞癌70例，混合型16例；分化程度：低分化96例，中、高分化86例；TNM临床分期：I~II期80例，III~IV期102例；有淋巴结转移104例，无淋巴结转移78例；有吸烟史132例，无吸烟史50例。每例患者另收集癌变组织旁5cm以外的癌旁组织作为对照组，每份标本均在30 min内置于－70℃的冰箱中保存待测。

1.2 实验方法

取50 mg左右冻存组织用于提取总RNA，先将组织标本剪碎，加入Trzol试剂1 mL，消化后用氯仿抽提，加入异丙醇提取总RNA，将提取的总RNA置于-70℃的冰箱中保存。应用MBI逆转录试剂盒（武汉华瑞康生物科技有限公司）合成cDNA，取3 μg总RNA进行逆转录，反应体系为20 μL，cDNA合成后置于-20℃的冰箱中保存。所有引物均由上海生工生物技术服务有限公司合成，具体如下：survivin上游引物：5'-CCACCGCATCTCTACATTCA-3'，下游引物：5'-TATGTTCCTCTATGGGGTCG-3'。Skp2上游引物：5'- AGTCTCTATGGCAGACCCTAGACC-3'，Skp2下游引物：5'-TTTCTGGAGATTCTTTCTGTAGCC-3'。XIAP上游引物：5'-AACCTTGTGATCGTGCCT-3'，下游引物：5'-ACCCTGGATACCATTTAGC- 3'。GAPDH的上游引物：5'- ATGACCACAGTCCATGCCAT-3'，下游引物：5'-TTCCTCTTGTGCTCTTGCTG-3'。聚合酶链反应（PCR）：取上述cDNA产物1 μL当作模板，GAPDH作为内参。实时定量PCR：在95 ℃下进行预变性，30 s；95 ℃下变性，5 s；在60 ℃下退火，31 s，重复40个循环。取10 μL PCR反应产物，进行1.5%琼脂糖凝胶电泳，采用溴化乙锭进行显色，通过凝胶成像系统对电泳结果进行分析。

1.3 统计学方法

选用SPSS19.0对所有数据进行统计分析，计数资料以率（%）表示，进行卡方检验，计量资料以均值± 标准差（$\bar{x}$± s）表示，进行t检验。以P<0.05为差异有统计学意义。

2 结果

2.1 Survivin、Skp2和XIAP mRNA在癌变组织和癌旁组织的表达

研究组的Survivin、Skp2和XIAP mRNA阳性率显著高于对照组，差异有统计学意义（P<0.05），具体见下表1。

表1 两组Survivin、Skp2和XIAP mRNA阳性率比较 [n(%)]

Table 1 Comparison of positive rate of Survivin, Skp2 and XIAP mRNA between two groups[n(%)]

Groups	n	Survivin mRNA		Skp2 mRNA		XIAP mRNA	
		Positive(+)	Negative(-)	Positive(+)	Negative(-)	Positive(+)	Negative(-)
Control group	182	26(14.29)	156(85.71)	31(17.03)	151(82.97)	25(13.74)	157(86.26)
Study group	182	134(73.63)	48(26.37)	130(71.43)	52(28.57)	110(60.44)	72(39.56)
x^2		130.076		109.157		85.069	
P		0.000		0.000		0.000	

2.2 研究组Survivin、Skp2和XIAP mRNA表达与临床病理特征的关系分析

Survivin mRNA的阳性表达与年龄、性别、组织类型、分化程度、临床分期、淋巴结转移、吸烟史无关（P>0.05）。Skp2 mRNA的阳性表达与年龄、性别、组织类型、分化程度、淋巴结转移、吸烟史无关（P>0.05），与临床分期相关（P<0.05）。XIAP mRNA的阳性表达与与年龄、性别、组织类型、分化程度、吸烟史无关（P>0.05），与临床分期、淋巴结转移相关（P<0.05），具体见下表。

3 讨论

恶性肿瘤对人类健康造成的负面影响仅次于心脑血管病，而其中肺癌的发病率、病死率最高[10]。在美国癌症协会2014年公布的调查结果中，在男性所有的恶性肿瘤疾病中肺癌的发病率仅在前列腺癌之后，女性中仅在乳腺癌之后，并且死亡率均排在首位[11,12]。随着医疗技术的不断发展，人类对于肿瘤的认识也逐渐从组织细胞水平深入到RNA、蛋白质水平。近年多项研究表明，Survivin、Skp2、XIAP、Caspase-9等基因通过异常表达而介导肿瘤的发生、发展过程报道表明，Survivin、Skp2、XIAP在恶性肿瘤的发生、发展过程中呈高表达水平，而Caspase-9通过低表达而参与肿瘤的发病过程低表达[13-15]。这种基因不同表达的状态组合成了具有肿瘤特征性的基因表达谱，可对转录和蛋白质的水平进行调控。恶性肿瘤的发展不仅与细胞增殖、分化程度相关，与细胞凋亡也密切相关[16,17]。细胞凋亡是指在基因调控下细胞有序死亡的过程，通过细胞凋亡可对体内衰老的细胞进行清理，配合细胞增殖可保持整体细胞的正常功能[18-20]。肿

瘤有两个发病途径，其一是细胞增殖过度，其二是细胞凋亡受阻，若肿瘤细胞中细胞凋亡受到影响，细胞过量增长，导致病变组织中的肿瘤细胞生存时间增加，破坏细胞群体内存活与死亡的平衡。因此如何减少细胞凋亡的抑制，防止细胞过度增殖成为治疗肿瘤新的切入点，而 Survivin、Skp2、XIAP 等具有凋亡抑制功能的基因也成为临床研究的重点。

表 2 Survivin、Skp2 和 XIAP mRNA 表达与临床病理特征的关系分析

Table 2 Relationship between expression of Survivin, Skp2, XIAP mRNA and clinicopathological features

Clinical indicators		n	Survivin mRNA		x^2	P	Skp2 mRNA		x^2	P	XIAP mRNA		x^2	P
			Positive (+)	Negative (-)			Positive (+)	Negative(-)			Positive (+)	Negative(-)		
Age(years)	60	112	80 (72.42)	32 (28.57)	0.724	0.395	75 (66.96)	37 (33.03)	2.844	0.092	65 (58.04)	47 (41.96)	0.704	0.402
	<60	70	54 (77.14)	16 (22.86)			55 (78.57)	15 (21.43)			45 (64.29)	25 (35.71)		
Gender	males	101	74 (73.27)	27 (26.73)	0.015	0.902	74 (73.27)	27 (26.73)	0.376	0.540	64 (63.37)	37 (36.63)	0.813	0.367
	femals	81	60 (74.07)	21 (25.93)			56 (69.14)	25 (30.86)			46 (56.79)	35 (43.21)		
Organization type	Adenocarcinoma	96	72 (75.00)	24 (25.00)	1.129	0.569	69 (71.88)	27 (28.13)	2.096	0.351	58 (60.42)	38 (39.58)	0.900	0.637
	Squamous cell carcinoma	70	52 (74.29)	18 (25.71)			52 (74.29)	18 (25.71)			44 (62.86)	26 (37.14)		
	Adenosquamous carcinoma	16	10 (62.50)	6(37.50)			9 (56.25)	7(43.75)			8 (50.00)	8 (50.00)		
Differentiation degree	poorly differentiation	96	76 (79.17)	20 (20.83)	3.211	0.073	70 (72.92)	26 (27.08)	0.220	0.639	62 (64.58)	34 (35.42)	1.459	0.227
	medium and highly differentiation	86	58 (67.44)	28 (32.56)			60 (69.77)	26 (30.23)			48 (55.81)	38 (44.19)		
Clinial stage	I-II	80	62 (77.50)	18(22.5)	1.103	0.294	50 (62.50)	30 (37.50)	5.576	0.018	39 (48.75)	41 (51.25)	8.158	0.004
	III-IV	102	72 (70.59)	30 (29.41)			80 (78.43)	22 (21.57)			71 (69.61)	31 (30.39)		
Lymph node metastasis	Yes	104	77 (74.04)	27 (25.96)	0.021	0.884	72 (69.23)	32 (30.77)	0.574	0.449	70 (67.31)	34 (32.69)	4.787	0.029
	No	78	57 (73.08)	21 (26.92)			58 (74.36)	20 (25.64)			40 (51.28)	38 (48.72)		
History of smoking	Yes	132	100 (75.76)	32 (24.24)	1.124	0.289	96 (72.73)	36 (27.27)	0.397	0.529	83 (62.88)	49 (37.12)	1.196	0.274
	No	50	34 (68.00)	16 (32.00)			34 (68.00)	16 (32.00)			27 (54.00)	23 (46.00)		

在本次研究中，研究组的 Survivin、Skp2 和 XIAP mRNA 阳性率显著高于对照组，差异有统计学意义(P<0.05)。这说明在 NSCLC 患者的癌变组织中 Survivin、Skp2 和 XIAP mRNA 呈现高表达，可能与疾病的发生、发展存在相关性。Survivin 是一种由 142 个氨基酸组成的凋亡抑制蛋白，以同源二聚体形式存在，分子量约为 16.5kD，是目前发现特异性最强的凋亡抑制因子之一[21,22]。多项研究发现，Survivin 在乳腺癌、肺癌、结肠癌、卵巢癌、前列腺癌等恶性肿瘤中呈现高表达，其表达的特异性在 100 多种肿瘤特异性表达基因中列于第 4 位[23,24]。另外本研究发现，Survivin mRNA 的阳性表达与年龄、性别、组织类型、分化程度、临床分期、淋巴结转移、吸烟史无关(P>0.05)，这与赵淑灿等人的研究结果一致[25]，但在 Zhang K 等人的研究中[26]，Survivin 与临床分期相关，造成差异的原因可能是多项研究的病例数存在一定差异。Skp2 基因定位于人 5 号染色体短臂上，分子质量约 45kD，能作用于磷酸化的细胞周期负性调控因子 p27kip1，使 p27kip1 通过泛素 - 蛋白酶体途径降解，引起细胞周期异常，Skp2 在多种恶性肿瘤中表达明显上升，主要包括乳腺癌、消化道肿瘤、肺癌等[27]。在本次研究中，Skp2 mRNA 的阳性表达与临床分期相关(P<0.05)，这与仇铁峰等人的研究结果一致[28]。XIAP 是凋亡抑制蛋白家族中最高效的天冬氨酸特异性半胱氨酸蛋白酶(Caspase)抑制剂之一，通过抑制 Caspase 来抑制细胞凋亡。相关研究发现，XIAP 抑制剂能够与肿瘤坏死因

子相关凋亡诱导配体(TRAIL)发生作用,增强 Caspase-3 活性,对胰腺癌细胞的增殖产生抑制作用,诱导胰腺癌细胞凋亡,且对正常细胞不产生影响[29]。在本次研究中,XIAP mRNA 的阳性表达与年龄、性别、组织类型、分化程度、吸烟史无关(P>0.05),与临床分期、淋巴结转移显著相关(P<0.05),这与叶闻远等人的研究结果一致[30],提示 XIAP 有可能参与了 NSCLC 的进展。

综上所述,在 NSCLC 患者的病理组织中,Survivin、Skp2 和 XIAP mRNA 的阳性率较高,且 Skp2mRNA 的阳性表达与临床分期有关,XIAP mRNA 的阳性表达与临床分期、淋巴结转移有关,Survivin、Skp2 和 XIAP 均可能参与了肿瘤的发生、发展,后期可对其进入更加深入的研究。

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