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临床研究 CLINICAL RESEARCH

HIF-1α及耐药蛋白在胰腺癌中的表达及相关性分析

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Expression and correlation of Hypoxia inducible Factor 1α and drug-resistance-correlated proteins in human pancreatic carcinoma

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Abstract

AIM: To investigate the expression of Hypoxia inducible Factor 1α(HIF-1α) and the drug-resistance-correlated indexes P-glycoprotein (P-Gp), Bcl-X_L and Bax, and the relationship between HIF-1α and resistance to chemotherapy in human pancreatic carcinoma.

METHODS: Protein expression of HIF-1α, P-Gp, Bcl-X_L and Bax in pancreatic tissues from 34 radically resected specimens of pancreatic carcinoma was detected by immunohistochemical staining.

RESULTS: The protein expression rates of HIF-1α, P-Gp, Bcl-X_L and Bax in pancreatic carcinoma were 26/34 (76.5%), 28/34 (82.4%), 30/34 (88.2%) and 26/34 (76.5%), respectively. There were positive correlations between the positivity rates of HIF-1α and P-Gp, Bcl-X_L and Bax (r = 0.471, P = 0.005; r = 0.443, P = 0.009; r = 0.510, P = 0.002).

CONCLUSION: HIF-1α, P-Gp, Bcl-X_L and Bax were over-expressed in human pancreatic carcinoma tissues. A positive correlation was determined between the expression rates of HIF-1α and P-Gp, Bcl-X_L and Bax. HIF-1α may have a significant role in resistance to chemotherapy in pancreatic carcinoma via up-regulation of the expression of P-Gp, Bcl-X_L and Bax.

Key Words: Pancreatic carcinoma; Hypoxia inducible Factor 1α; Chemotherapy resistance; Multidrug resistance gene; Apoptosis

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摘要

目的: 探讨HIF-1α及耐药相关指标P糖蛋白(P-Gp), 凋亡相关Bcl-2家族成员Bcl-X_L及Bax在人胰腺癌组织中表达的相关性.

方法: 免疫组化法检测手术切除的胰腺癌组织34例中HIF-1α、P-Gp、Bcl-X_L、Bax蛋白表达.

结果: 34例胰腺癌标本中有26例(76.5%)HIF-1α, 28例(82.4%)P-Gp, 30例(88.2%)Bcl-X_L, 26例(76.5%)Bax表达阳性. HIF-1α和P-Gp、Bcl-X_L、Bax蛋白表达呈正相关(r = 0.471, P = 0.005; r = 0.443, P = 0.009; r = 0.510, P = 0.002).

结论: 在胰腺癌组织中HIF-1α、P-Gp、Bcl-X_L、Bax蛋白存在高表达; HIF-1α与P-Gp、Bcl-X_L、Bax的蛋白表达呈正相关, 提示HIF-1α可能通过上调耐药相关蛋白P-Gp、Bcl-X_L及Bax蛋白表达在胰腺癌耐药机制中起重要作用.

关键词: 胰腺癌; 缺氧诱导因子-1α; 化疗耐药; 多药耐药基因; 凋亡

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■背景资料
胰腺癌是1种恶性程度极高的消化道肿瘤, 在上海, 其发病率近20 a内增加近4倍, 上升至第9位, 并呈逐年上升趋势. 在美国胰腺癌的发病率同样上升至第4位, 每年约有37 170例胰腺癌新发病例, 而同期死于胰腺癌的患者约有33 370例.

■相关报道
对于实体肿瘤微环境缺氧与耐药相关机制的研究在国内外都有相关报道, 国内以上海交通大学附属第一人民医院普外科及华中科技大学同济医学院的研究报道较多, 可供广大读者参考.

0 引言

胰腺癌的恶性程度高[1], 早期诊断困难[2-3], 发病率呈逐年上升趋势[4-7], 手术切除率低[8-9], 化疗效果差, 即使是二氟胞苷(吉西它滨, gemcitabine), 其有效率也仅为12%[10-11]. 近年来胰腺癌化疗耐药机制已经成为研究热点, 影响肿瘤化疗耐药因素较多, 其中缺氧微环境在肿瘤化疗中的作用越来越受到研究者的重视, 实体瘤存在不同程度的缺氧, 缺氧可诱导核转录因子-缺氧诱导因子-1(hypoxia-inducible factor -1, HIF-1)表达增高[12-13], 从而上调下游靶基因的表达, 其中也有多药耐药基因1(multidrug resistance gene1, mdr1)编码的膜蛋白P糖蛋白(P-glycoprotein, P-Gp)以及凋亡相关Bcl-2家族成员Bcl-X_L和Bax[14]. 我们应用免疫组化方法检测了人胰腺癌组织中HIF-1α及耐药相关蛋白的表达, 旨在探讨这些蛋白在胰腺癌中的表达情况, 及HIF-1α与耐药相关蛋白的关系.

1 材料和方法

1.1 材料 1998-01/2006-02手术切除后病理证实为胰腺导管腺癌患者34例, 男22例, 女12例, 年龄35-76(平均59.7±10.8)岁. TMN(UICC, 2002) Ⅰ期3例, Ⅱ期16例, Ⅲ期9例, Ⅳ期6例. 高分化18例, 中分化14例, 低分化2例. 胰头癌30例, 胰体尾部癌4例. 所有标本经甲醛固定, 石蜡包埋切片2 μm, 进行免疫组化染色. 兔抗人HIF-1α mAb(sc-10790)、鼠抗人P-Gp mAb(sc-13131)、鼠抗人Bcl-X_L mAb(sc-8392)、兔抗人Bax mAb(sc-526)购自美国Santa Cruz生物技术有限公司, 二抗(D-3004)购自上海长岛生物技术有限公司.

1.2 方法 采用二步法免疫组化染色(EnVision System). 常规石蜡包埋的组织连续切片; 二甲苯脱蜡, 梯度乙醇逐级水化; 30mL H_2O_2甲醇溶液灭活内源性过氧化物酶; 微波抗原修复; 羊血清封闭非特异性抗原, 加入一抗HIF-1α(1∶100), P-Gp(1∶100), Bcl-X_L(1∶200)和Bax(1∶200), 4℃ 30min; 加入EnVision二抗室温下孵育30 min; 加入DAB显色, 光镜控制显色程度; 苏木素复染, 中性树胶封片. 以公司提供阳性对照片为阳性对照, 以PBS代替一抗作为阴性对照. 参考文献[15-18]判断HIF-1α, P-Gp, Bcl-X_L和Bax结果.

统计学处理 采用SPSS10.0统计软件进行分析, 计数资料用χ^2检验或四格表精确概率检验及Spearman等级相关检验作统计分析, P<0.05有统计学意义.

表 1 胰腺癌HIF-1α、P-Gp、Bcl-X_L、Bax在细胞中表达的关系

HIF-1α	P-Gp		Bcl-X_L		Bax	
	(+)	(–)	(+)	(–)	(+)	(–)
(+)	24[b1]	2	25[b2]	1	23[b3]	3
(–)	4	4	5	3	3	5
合计	28	6	30	4	26	8

[b1]P = 0.005 (r = 0.471), [b2]P = 0.009 (r = 0.443), [b3]P = 0.002 (r = 0.510).

2 结果

2.1 胰腺癌组织HIF-1α及P-Gp、Bcl-X_L、Bax的表达 34例胰腺癌组织中HIF-1α表达阳性26例(76.5%), P-Gp表达阳性28例(82.4%), Bcl-X_L表达阳性30例(88.2%), Bax表达阳性26例(76.5%). HIF-1α阳性的细胞在胰腺癌组织中呈散在、局灶性分布, 尤其在肿瘤组织内的腺管结构中及坏死组织周围表达较高, 染色颗粒主要位于胰腺癌细胞的胞核内; P-Gp阳性的细胞在胰腺癌组织中呈散在、局灶或弥漫性分布, 染色颗粒主要位于胰腺癌细胞的胞膜上与胞质内; Bcl-X_L、Bax阳性的细胞在胰腺癌组织中呈弥漫性分布, 染色颗粒主要位于胰腺癌细胞的胞质内(图1).

2.2 HIF-1α与P-Gp, Bcl-X_L, Bax表达的关系 HIF-1α阳性26例中有24例P-Gp表达阳性, HIF-1α阴性8例中有4例P-Gp阳性, HIF-1α和P-Gp蛋白表达呈正相关(r = 0.471, P = 0.005, 表1). HIF-1α和Bcl-X_L, Bax蛋白表达呈正相关(表1).

3 讨论

实体肿瘤存在不同程度的低氧状态[19], 目前已有研究表明肿瘤低氧微环境可降低化疗的敏感性[20-22], 进一步的实验研究表明低氧可诱导多种肿瘤细胞株对抗癌药物的耐药[23]. Buchler *et al*[24]检测了胰腺癌组织中的氧分压, 发现胰腺癌组织存在低氧微环境. 有研究发现, 胰腺癌组织中存在HIF-1α高表达, 并与肿瘤大小、临床病理分期和淋巴结转移有关[25]. 本研究进一步证实了胰腺癌组织中存在HIF-1α的高表达. 低氧微环境导致肿瘤对化疗药物耐药的机制仍不清楚, 近年的研究表明低氧微环境可诱导耐受低氧诱导凋亡细胞克隆[26], 并且HIF-1α在这一过

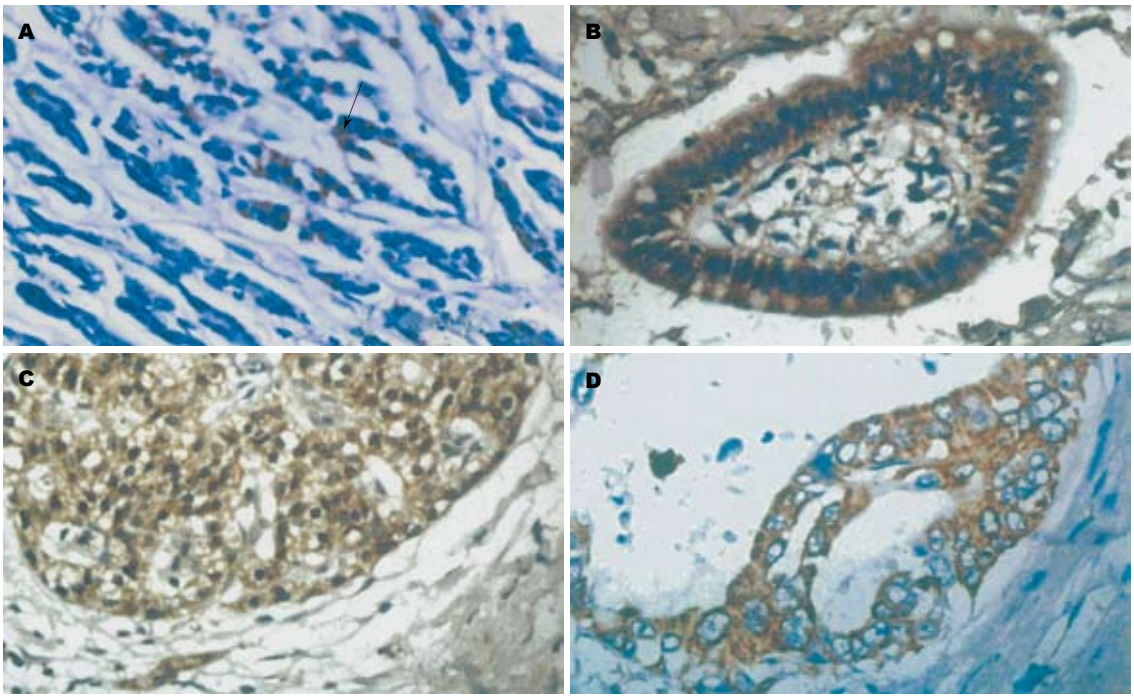

图 1 胰腺癌组织中HIF-1α, P-Gp, Bcl-X_L, Bax蛋白的表达(EnVision × 400). A: HIF-1α; B: P-Gp; C: Bcl-X_L; D: Bax.

程中起重要作用, HIF-1α可上调抗凋亡基因表达, Acs *et al*[27]认为缺氧能诱导Bcl-X_L过表达抑制凋亡. Ozawa *et al*[28]则发现缺氧条件下, HIF-1的激活能引起Bax蛋白的表达增加从而启动Bax介导的细胞凋亡. 本研究从临床病理组织中发现并证实胰腺癌组织中存在Bcl-X_L和Bax的高表达, 并且HIF-1α高表达与Bcl-X_L和Bax表达呈正相关, 提示低氧微环境下HIF-1α可能通过上调Bcl-X_L和Bax的表达使胰腺癌细胞耐受凋亡, 可能是胰腺癌细胞对化疗耐药的机制之一.

低氧微环境也可能通过调控MDR1/P-Gp表达而产生耐药, Waternberg *et al*[29]在体外研究中发现低氧微环境可上调P-Gp表达, 并且需要HIF-1α的参与. 进一步用反寡核苷酸阻断HIF-1α表达可导致MDR1 mRNA和P-Gp的上调[30], 而且低氧微环境诱导HIF-1α、P-Gp表达上调, JNK激酶传导信号起重要作用[31]. 本研究发现胰腺癌组织中存在P-Gp高表达, 并且与HIF-1α表达呈正相关, 提示MDR1可能是HIF-1α的靶基因之一.

本研究从免疫组化上发现胰腺癌组织中存在HIF-1α、P-Gp、Bcl-X_L和Bax的高表达, 并且P-Gp、Bcl-X_L和Bax的表达与HIF-1α呈正相关, HIF-1α可能通过上调P-Gp、Bcl-X_L和Bax, 在胰腺癌耐药机制中起重要作用, 其确切机制还有待于进一步深入研究.

4 参考文献

1 Boeck S, Hinke A, Wilkowski R, Heinemann V. Importance of performance status for treatment outcome in advanced pancreatic cancer. *World J Gastroenterol* 2007; 13: 224-227
2 Boujaoude J. Role of endoscopic ultrasound in diagnosis and therapy of pancreatic adenocarcinoma. *World J Gastroenterol* 2007; 13: 3662-3666
3 Iglesias-Garcia J, Dominguez-Munoz E, Lozano-Leon A, Abdulkader I, Larino-Noia J, Antunez J, Forteza J. Impact of endoscopic ultrasound-guided fine needle biopsy for diagnosis of pancreatic masses. *World J Gastroenterol* 2007; 13: 289-293
4 Chen KX, Wang PP, Zhang SW, Li LD, Lu FZ, Hao XS. Regional variations in mortality rates of pancreatic cancer in China: results from 1990-1992 national mortality survey. *World J Gastroenterol* 2003; 9: 2557-2560
5 Wang L, Yang GH, Lu XH, Huang ZJ, Li H. Pancreatic cancer mortality in China (1991-2000). *World J Gastroenterol* 2003; 9: 1819-1823
6 陈可欣, 张思维, 李连弟. 中国胰腺癌死亡情况报告. 中华流行病学杂志 2003; 24: 520-522
7 Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007; 57: 43-66
8 Nakao A, Fujii T, Sugimoto H, Kanazumi N, Nomoto S, Kodera Y, Inoue S, Takeda S. Oncological problems in pancreatic cancer surgery. *World J Gastroenterol* 2006; 12: 4466-4472
9 Wray CJ, Ahmad SA, Matthews JB, Lowy AM. Surgery for pancreatic cancer: recent controversies and current practice. *Gastroenterology* 2005; 128: 1626-1641
10 Xie DR, Liang HL, Wang Y, Guo SS, Yang Q. Meta-analysis on inoperable pancreatic cancer: a comparison between gemcitabine-based combination therapy and gemcitabine alone. *World*

■创新盘点
本文以胰腺癌临床病理标本为研究对象, 应用免疫组化的方法研究胰腺癌组织中缺氧及耐药相关基因蛋白水平的表达, 为微环境缺氧在胰腺癌治疗领域中的应用提供临床依据.

■应用要点
本文为靶向微环境缺氧治疗胰腺癌化疗耐药提供了初步的临床理论依据, 虽然靶向微环境缺氧的治疗真正应用于临床可能还需要经过漫长的临床试验过程, 但是他为胰腺癌的治疗提供了新的思路.

■同行评价
本文通过免疫组化方法分析胰腺癌组织中HIF及相关耐药蛋白的表达, 发现胰腺癌组织中存在HIF, Bcl-X_L和Bax的高表达, 选题明确, 有一定针对性, 可为临床胰腺癌化疗耐药研究提供新靶点, 有较好学术价值.

J Gastroenterol 2006; 12: 6973-6981
11 Storniolo AM, Enas NH, Brown CA, Voi M, Rothenberg ML, Schilsky R. An investigational new drug treatment program for patients with gemcitabine: results for over 3000 patients with pancreatic carcinoma. *Cancer* 1999; 85: 1261-1268
12 Alvarez-Tejado M, Alfranca A, Aragones J, Vara A, Landazuri MO, del Peso L. Lack of evidence for the involvement of the phosphoinositide 3-kinase/Akt pathway in the activation of hypoxia-inducible factors by low oxygen tension. *J Biol Chem* 2002; 277: 13508-13517
13 Caro J. Hypoxia regulation of gene transcription. *High Alt Med Biol* 2001; 2: 145-154
14 Longley DB, Johnston PG. Molecular mechanisms of drug resistance. *J Pathol* 2005; 205: 275-292
15 Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW. Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. *Cancer Res* 1999; 59: 5830-5835
16 Weinstein RS, Jakate SM, Dominguez JM, Lebovitz MD, Koukoulis GK, Kuszak JR, Klusens LF, Grogan TM, Saclarides TJ, Roninson IB. Relationship of the expression of the multidrug resistance gene product (P-glycoprotein) in human colon carcinoma to local tumor aggressiveness and lymph node metastasis. *Cancer Res* 1991; 51: 2720-2726
17 Kanczuga-Koda L, Sulkowski S, Koda M, Skrzydlewska E, Sulkowska M. Connexin 26 correlates with Bcl-xL and Bax proteins expression in colorectal cancer. *World J Gastroenterol* 2005; 11: 1544-1548
18 Miyamoto Y, Hosotani R, Wada M, Lee JU, Koshiba T, Fujimoto K, Tsuji S, Nakajima S, Doi R, Kato M, Shimada Y, Imamura M. Immunohistochemical analysis of Bcl-2, Bax, Bcl-X, and Mcl-1 expression in pancreatic cancers. *Oncology* 1999; 56: 73-82
19 Shannon AM, Bouchier-Hayes DJ, Condron CM, Toomey D. Tumour hypoxia, chemotherapeutic resistance and hypoxia-related therapies. *Cancer Treat Rev* 2003; 29: 297-307
20 Durand RE. The influence of microenvironmental factors during cancer therapy. *In Vivo* 1994; 8: 691-702
21 Raghunand N, Gillies RJ. pH and drug resistance in tumors. *Drug Resist Updat* 2000; 3: 39-47
22 Dong Z, Wang JZ, Yu F, Venkatachalam MA. Apoptosis-resistance of hypoxic cells: multiple factors involved and a role for IAP-2. *Am J Pathol* 2003; 163: 663-671
23 Volm M, Koomagi R, Mattern J. Interrelationships between microvessel density, expression of VEGF and resistance to doxorubicin of non-small lung cell carcinoma. *Anticancer Res* 1996; 16: 213-217
24 Buchler P, Reber HA, Buchler M, Shrinkante S, Buchler MW, Friess H, Semenza GL, Hines OJ. Hypoxia-inducible factor 1 regulates vascular endothelial growth factor expression in human pancreatic cancer. *Pancreas* 2003; 26: 56-64
25 Sun HC, Qiu ZJ, Liu J, Sun J, Jiang T, Huang KJ, Yao M, Huang C. Expression of hypoxia-inducible factor-1 alpha and associated proteins in pancreatic ductal adenocarcinoma and their impact on prognosis. *Int J Oncol* 2007; 30: 1359-1367
26 Sanna K, Rofstad EK. Hypoxia-induced resistance to doxorubicin and methotrexate in human melanoma cell lines in vitro. *Int J Cancer* 1994; 58: 258-262
27 Acs G, Chen M, Xu X, Acs P, Verma A, Koch CJ. Autocrine erythropoietin signaling inhibits hypoxia-induced apoptosis in human breast carcinoma cells. *Cancer Lett* 2004; 214: 243-251
28 Ozawa T, Hu JL, Hu LJ, Kong EL, Bollen AW, Lamborn KR, Deen DF. Functionality of hypoxia-induced BAX expression in a human glioblastoma xenograft model. *Cancer Gene Ther* 2005; 12: 449-455
29 Wartenberg M, Ling FC, Muschen M, Klein F, Acker H, Gassmann M, Petrat K, Putz V, Hescheler J, Sauer H. Regulation of the multidrug resistance transporter P-glycoprotein in multicellular tumor spheroids by hypoxia-inducible factor (HIF-1) and reactive oxygen species. *FASEB J* 2003; 17: 503-505
30 Comerford KM, Wallace TJ, Karhausen J, Louis NA, Montalto MC, Colgan SP. Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene. *Cancer Res* 2002; 62: 3387-3394
31 Comerford KM, Cummins EP, Taylor CT. c-Jun NH2-terminal kinase activation contributes to hypoxia-inducible factor 1alpha-dependent P-glycoprotein expression in hypoxia. *Cancer Res* 2004; 64: 9057-9061

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