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The role of GPC5 in lung metastasis of salivary adenoid cystic carcinoma

Yanning Zhang [a,c], Jie Wang [a,c,*], Fusheng Dong [b,c], Hexiang Li [a,c], Yali Hou [a,c]

[a] Department of Oral Pathology, College of Stomatology, Hebei Medical University, Shijiazhuang, PR China
[b] Department of Oral and Maxillofacial Surgery, College of Stomatology, Hebei Medical University, Shijiazhuang, PR China
[c] The Key Laboratory of Stomatology, No. 383 East Zhongshan Road, Shijiazhuang 050017, Hebei, PR China

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ABSTRACT

Objective: Proteoglycans play a crucial role in salivary adenoid cystic carcinoma (SACC) tumorigenesis, neurotropic growth and lung metastasis. In this study, we investigated the role of GPC5 in the lung metastasis of SACC.

Design: The expression of heparan sulfate proteoglycans (HSPGs) was detected in SACC-M (high lung-metastatic cell line), SACC-2 (low lung-metastatic cell line) and SACC-83 (low lung-metastatic cell line) cells by relative quantitative Real-Time PCR. The expression of GPC5 was detected by immunofluorescence and Western blot analysis in SACC-M, SACC-2 and SACC-83 cells, and by immunohistochemical analysis in primary tumours from 16 cases of SACC patients with or without lung metastasis. GPC5 expression was silenced in SACC-M cells, cell proliferation and tumour growth was evaluated by MTT assay and nude mice model, respectively.

Results: Expression of most HSPGs was decreased in SACC-M cells, but GPC5 expression increased 3.24-fold and 815.69-fold (more than 3-fold) in SACC-M compared with SACC-2 and SACC-83 cells, respectively. Immunohistochemical analysis showed higher expression of GPC5 in SACC with lung metastasis compared to SACC without lung metastasis. The lung metastasis of SACC-M cells in nude mice was obviously decreased after GPC5 silencing ($P < 0.05$), although GPC5 silencing had no significant effect on SACC-M cell proliferation.

Conclusion: GPC5 may contribute to lung metastasis of SACC.

1. Introduction

Salivary adenoid cystic carcinoma (SACC) is a common malignancy of salivary glands, accounting for the most cases of minor salivary gland malignancies and a substantial proportion of parotid and submandibular gland malignancies. SACC has a high propensity for perineural invasion and distant metastasis clinically, and the most common site of

* Corresponding author at: Department of Oral Pathology, College of Stomatology, Hebei Medical University, No. 383 East Zhongshan Road, Shijiazhuang 050017, Hebei, PR China. Tel.: +86 311 86265780; fax: +86 311 86051304.
E-mail addresses: wangjiephd@163.com, wangjiephd2010@tom.com (J. Wang).

Abbreviations: ECM, extra cellular matrix; GPC, glypican; HSPGs, heparan sulphate proteoglycans; SDC, syndecan; SACC, salivary adenoid cystic carcinoma; RNAi, RNA interference.

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distant metastases is the lung.[1] Our previous studies have indicated that proteoglycans play an important role in SACC proliferation, migration, metastasis and perineural invasion.[2,3]

Proteoglycans are widely expressed in salivary gland tumours,[4,5] and heparan sulfate proteoglycans (HSPGs) are present on the inner surfaces of true ductal spaces and pseudocysts of adenoid cystic carcinomas.[6,7] HSPGs have been shown to be involved in cell proliferation and characteristic histological structure of SACC.[8]

HSPGs, consisted of a protein core to which heparan sulphate glycosaminoglycan chains are attached, are abundant in cells and extracellular matrix (ECM). Secreted HSPGs include perlecan, agrin and type XVIII collagen. Syndecans and glypicans represent two major membrane HSPGs, four separate syndecan (SDC1, SDC2, SDC3, and SDC4) and six separate glypican (GPC1, GPC2, GPC3, GPC4, GPC5, and GPC6) have been identified in mammals. The syndecans have a transmembrane and cytoplasmic domain, whereas the glypicans are anchored to the extracytoplasmic face of the plasma membrane via glycosylphosphatidylinositol (GPI). HSPGs have been shown to act as receptors or co-receptors and play a central role in many biological processes, such as cell proliferation, cell adhesion/anti-adhesion, chemo-attraction, inflammation, wound healing, coagulation, matrix assembly, embryo development, and tumour metastasis.[9]

The amino acid sequences of the six vertebrate glypican family members are 17% to 63% identical, and GPC3 is the most homologous to GPC5.[10] GPC5 gene has eight exons encoding 572 amino acids and spans a large genomic region of 1.47 Mb at chromosome 13q31.3.[11] The amplification of 13q31–32 is frequently observed in a broad range of tumour types, including rhabdomyosarcomas, liposarcomas with poor prognosis, lymphomas, lung carcinomas, breast cancers and medulloblastoma.[12–19] GPC5 is expressed mainly in the developing central nervous system, the limbs, the kidney, the lung and the liver. In adults, GPC5 is primarily expressed in brain tissue.

In present study, we detected the expression of two major membrane HSPGs and an extracellular HSPG (perlecan) in different SACC cell lines with high or low lung-metastatic potential. In particular, we compared the expression of GPC5 in primary tissues from SACC patients with or without lung metastasis to investigate the role of GPC5 in lung metastasis. Furthermore, we employed RNA interference (RNAi) to silence the expression of GPC5 in SACC-M (SACC cell line with high lung-metastatic potential) cells,[20] and observe the effect on lung metastasis in nude mice.

2. Materials and methods

2.1. Cell culture

Human SACC cell lines SACC-M and SACC-2[21] were previously established by Tumour Biology Laboratory of Shanghai Ninth Hospital in Shanghai Second Medical University. SACC-83[22] cell line was established by the Laboratory of Oral and Maxillofacial Surgery, Health Science Center, Peking University. The cells were routinely cultured in RPMI-1640 medium (Hyclone, USA) supplemented with 15% foetal bovine serum (Gibco, USA), penicillin (100 U/ml), and streptomycin (100 U/ml) at 37 °C in humanized air containing 5% CO_2.

Cell line SACC-2 was derived from surgically excised primary tumour tissue from a histologically diagnosed patient with adenoid cystic carcinoma of the palate.[21] High lung metastases clone SACC-M was selected from cell line ACC-2 after five repeated selection in vivo combined with an in vitro cloning technique and analysis of platelet aggregation activity. Compared with SACC-2, its metastatic rate was 96% vs. 18%.[20] SACC-83 was established from a patient pathologically diagnosed as SACC in sublingual gland and it has a relatively low lung-metastatic rate.[22,23]

2.2. Real-time PCR

Total RNA was extracted from the cells by using TRIzol reagent (Invitrogen, USA) following the manufacturer's instructions. RNA concentration was analyzed based on spectrophotometric readings at 260 and 280 nm, and quality was evaluated by gel electrophoresis. Validated RNA samples were stored at −80 °C, and 3 μg of total RNA from each sample were reverse transcribed by using RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Lithuania) at 42 °C for 1 h, and 70 °C for 5 min.

Real-Time PCR assay was performed by using 7500 Real-Time PCR System (Applied Biosystems, USA). The house keeping gene GAPDH was used as reference gene. Real-Time PCR was carried out according to the instructions for Power SYBR Green PCR Master Mix (Applied Biosystems, USA). The forward and reverse primers were shown in Table 1. The parameters were as following: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles at 95 °C for 20 s, 57 °C for 20 s and 72 °C for 31 s.

Table 1 – Primer sequences of HSPGs and GAPDH.

Gene	Sequence (5′–3′)
SDC1	f: CTACTAATTTGCCCCCTGAAGA, r: GGTCTGCTGTGACAAGGTGATA
SDC2	f: CAGCCGAAGAGGATACAAATG, r: CGATACACCAACAGCAGGATAA
SDC3	f: TTCTTGGTCACACTGCTCATCT, r: CTTGTCAGGCTTCTGGTATGTG
SDC4	f: AAGGTGTCAATGTCCAGCACT, r: GGGCTTTCTTGTAGATGGGTTT
perlecan	f: GCCAGCGTGGGACTTAGCGACAT, r: CGCAGGACAAGCCAGAATAGCC
GPC1	f: CAGAGCAGGAAGGACAGAAGAC, r: CTACTGTAAGGGCCAGGAAGAG
GPC2	f: CTTCTATGGGGAATCTGGTGAG, r: GTGAGAGGCAGAGCAGGTAGTC
GPC3	f: GGCTCTGGTGATGGAATGATA, r: GAGTTGCCTGCTGACTGTTTC
GPC4	f: TCGTTCCATCTCTGAAAGTGC, r: TTTCCCATTCCAACAGTCATC
GPC5	f: AGACCACCACAAGGAACAGTG, r: AGACTGGGCTTTGATTCCATT
GPC6	f: AGGTCTTTCAGGGATGTGGTC, r: GCAGTTGTTGGTCTTTCCTCA
GAPDH	f: CATGAGAAGTATGACAACAGCCT, r: AGTCCTTCCACGATACCAAAGT