Role of mTOR/P70 S6K Signaling in Oral Acinic Cell Carcinoma

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Abstract **Purpose** To observe the expression of P70 S6 kinase (P70 S6K) signal pathway in oral acinic cell carcinoma. **Methods** P70 S6 kinase were examined by means of Western-blot test and Activity Assay in 30 cases of oral acinic cell carcinoma. **Results** The expression level of the Western-blot of p70 S6 k increase obviously in oral acinic cell carcinoma tissue ($P<0.01$). Activity assay is the same as the Western-blot test ($P<0.01$). P70 S6K expression and activity play an important role in development of oral acinic cell carcinoma. **Conclusion** The overexpression of P70 S6K may be involved in the pathogenesis of oral acinic cell carcinoma, and the inhibition of its expression may be a therapeutic way for cancer patients.

Key Words p70 S6K; acinic cell carcinoma; western-blot

Mammalian target of rapamycin (mTOR) is one member of the recently identified family of protein kinase termed phosphoinositide 3-kinase related kinases (PIKKs) which are involved in many critical regulatory cellular functions pertaining to cell cycle progression, cell cycle checkpoints that govern cellular response to DNA damage, DNA repair, and DNA recombination. mTOR is a central controller of cell growth[1]. P70 S6K (the 40S ribosomal protein S6 kinase) is the downstream target of mTOR. mTOR phosphorylates p70 S6k on Thr-389, a residue whose phosphorylation is rapamycin-sensitive in vivo and necessary for S6 kinase activity. In turn, P70 S6K phosphorylates the 40S ribosomal protein S6 (S6). The phosphorylation of S6 leads to the recruitment of the 40S ribosomal subunit into actively translating polysomes, thereby enhancing the translation of mRNAs with a 5′ terminal oligopolypyrimidine[2].

Acinic cell carcinoma is one of common cancers among men. P70 S6K probably plays an important regulating role in the appearance of acinic cell carcinoma. But nothing about this aspect has been reported yet. So we choose acinic cell carcinoma as the subject of our research, and study the expression of P70 S6K in oral acinic cell carcinoma tissue and the pathogenesis of oral acinic cell carcinoma.

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MATERIALS AND METHODS

Tissue specimens

The tissue specimens were from the Maxillofacial surgical Department of College of Stomatology, China Medical University from 2001 to 2003, which included 30 samples of oral acinic cell carcinoma and 15 samples of normal oral tissue used as control.

The main reagents

P70 S6 kinase、Peroxidase-Conjugated Goat Anti-Rabbit IgG (H+L)、ECL Plus Western bloting detection system were purchased from Santa Cruz Biotechnology Inc. Protein Assay ESL、Bench-MarkTM protein Ladder were purchased from GIBCO.

Methods

Western Blotting Frozen oral acinic cell carcinoma tissue and oral normal tissue were grounded in liquid nitrogen by mortar and pestle and tissue powder was washed with PBS and lysed at 4℃

with 25 mM Tris·HCl, pH 7.4/50 mM NaCl/0.5% sodium deoxycholate/2% Nonidet P-40 (NP40)/0.2% SDS/1 μM phenylmethylsulfonyl fluoride (PMSF)/50 μg/ml aprotinin/50 μM leupeptin. Lysates were resolved by SDS/8% polyacrylamide gels and transferred to nitrocellulose filters. Specific reactive proteins were detected by the enhanced chemiluminescence (ECL) method, employing a sheep anti-mouse IG antibody linked to horseradish peroxidase.

S6K Activity Assay The specific activities of p70 S6K were determined by ^{32}P incorporation into S6 peptide in the immune complex as described previously[3]. Briefly, tissue fragments were lysed at 4℃ in 500 μl of lysis buffer (10 mM potassium phosphate/1 mM EDTA/5 mM EGTA/10 mM MgCl2/50 mM β-glycerophosphate/1 mM Na_3VO_4/2 mM DTT/40 μg/ml PMSF/0.1% NP40). The extract was incubated for 30 min at 4℃ with the anti–P70 S6K immunocomplex. The immune complex was absorbed to Protein G-coupled beads for 30 min and washed twice with the lysis buffer and once with kinase buffer (20 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$/1 μg/ml IP-20/0.1 mg/ml BSA/0.4 mM DTT). After the final wash, the immune complexes were suspended in 50 μl of the kinase buffer containing 100 μM unlabeled ATP, 200 μCi/ml γ-^{32}P ATP (1 Ci = 37 GBq), and 125 μM S6 peptide (RRRLSSLRA). The reaction was allowed to proceed for 15 min at 30℃ and terminated by the addition of 20 μl of 250 mM EDTA and boiling for 5 min. After a brief centrifugation, the supernatant (25 μl) was applied to phosphocellulose paper and radioactivity was determined by using a liquid scintillation counter[4].

RESULTS

Western blot of P70 S6K in normal oral tissue and acinic cell carcinoma tissue

Western blot show that the expression of P70 S6K in acinic cell carcinoma tissue increased compared with that of normal tissue. By grey scale analysis, the expression of P70 S6K in acinic cell carcinoma tissue is 1.58 times higher than that of normal tissue (Figs.1, 2), the difference is remarkable (P<0.01).

Densitometric

P70 S6 kinase activity assay in normal oral tissue and acinic cell carcinoma tissue

S6K kinase activity was elevated remarkably in acinic cell carcinoma tissue compared with normal tissue. By grey scale analysis, the expression of P70 S6K in acinic cell carcinoma tissue is 2.12 times higher than that of normal tissue (Figs.3, 4), the difference is remarkable (P<0.01).

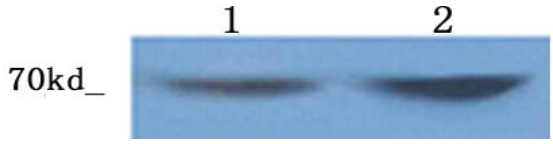

Fig.1 Western blot analysis of P70 S6K using antibody against P70 S6K. 1. control; 2. acinic cell carcinoma tissue

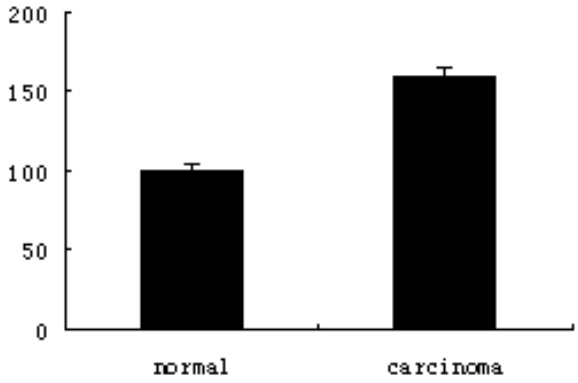

Fig.2 Western blot analysis of P70 S6K using antibody against P70 S6K. 1. control; 2. acinic cell carcinoma tissue

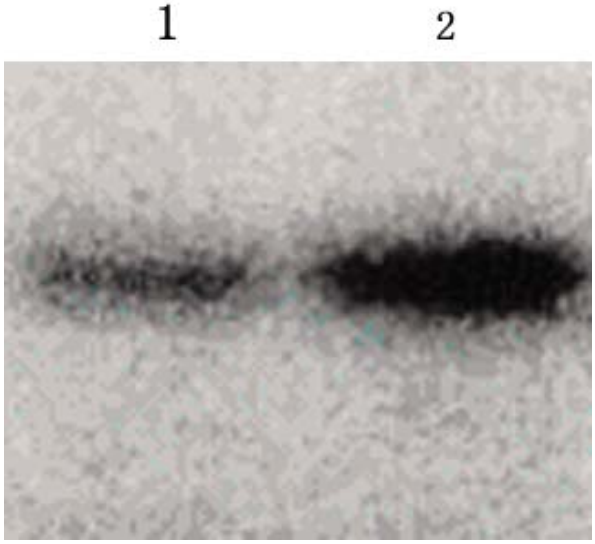

Fig.3 Activation of P70 S6K in normal tissue and acinic cell carcinoma tissue. 1. normaltissue 2. acinic cell carcinoma tissue

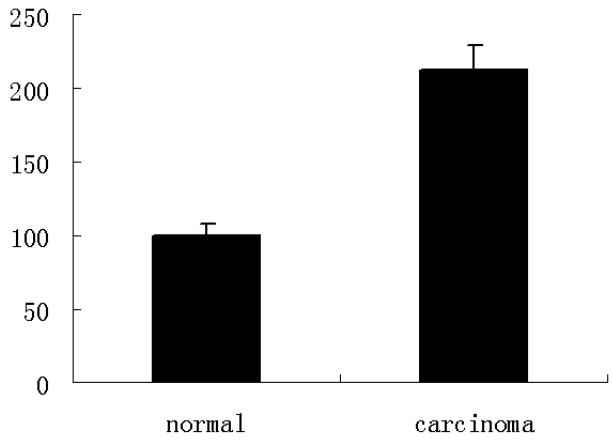

Fig.4 Western blot analysis of P70 S6K using antibody against P70 S6K.
1. control;
2. acinic cell carcinoma tissue

DISCUSSION

The growth of a cell is one of the premises of the proliferation of the cell, because the cell must grow to a certain level before the cell divide. The finding of mTOR put the study in the field of the cell growth and proliferation to a new stage [5]. As the study goes further, the important role played by S6K, one of the downstream of mTOR in the process of cell growth appears. As a simple kinase, S6K can affect the translation of hundreds of mRNA, by regulating the 40S ribosomal protein S6 and then regulateing the biosynthesis. The experiment proved that S6K acted a decisive part in the growth of cell skeleton. The figure of young mouse with it S6K gene removed will apparently becomes small [6]. The cell growth and proliferation are two very closely related processes, the role of the S6K played in the cell cycle can not be neglected out. Deletion of the p70 S6K gene was achieved in ES cells by homologous recombination to define the function of p70 S6K in mammalian cells. Targeted disruption of the p70 S6K gene eliminated phosphorylation of ribosomal S6 protein and abrogated translational regulation of mRNAs encoding ribosomal proteins by serum and rapamycin in these murine ES cells. These data demonstrate that the function of p70 S6K resides in S6 phosphorylation and also in regulation of ribosomal protein synthesis at the level of mRNA translation. Ribosomal proteins are needed in equimolar amounts in ribosomal biogenesis[7]. So now S6K itself can be viewed as a bottleneck which can regulate growth. It is likely the key regulating factor of cell growth [8].

The high frequency of mutations in cancer cells which result in altered cell cycle regulation and growth signal transduction, conferring a proliferative advantage, indicates that many of these aberrant mechanisms may be the strategic targets to cancer therapy. It is clear that defining the molecular characteristics of tumors in clinical may help to identify which patients may benefit from treatment. Acinic cell carcinoma is one of the malignant tumors that seriously threat human life. The reasons contributed to the development of acinic cell carcinoma are very complicated, which involv different genes at different stages [9]. P70 S6K is the key signal molecule that regulates and controls the synthesis of protein. So P70 S6K signal pathway has played a regulating and controlling role in the development of acinic cell carcinoma. P70 S6k as a potential target for therapeutic strategies to preventing or inhibiting tumor growth[10].

In this experiment we choose the acinic cell carcinoma as the subject of study, to research the expression of P70 S6K in acinic cell carcinoma. The results showed a high expression level of P70 S6K in the acinic cell carcinoma, which indicated that P70 S6K is closely related with the pathogenesis of acinic cell carcinoma and inhibition of these proteins may be a therapeutic way for cancer patients.

REFERENCES

1. Schmelzle T, Hall M N. TOR,a Central Controller of Cell Growth. Cell, 2000, 103: 253–262.
2. Jefferies HB, Reinhard C, Kozma SC. Rapamycin selectively represses translation of the polypyrimidine tract mRNA family. Proc Natl Acad Sci USA, 1994, 91:4441–4445
3. Terada N, Takase K, Papst, P, et al. Rapamycin inhibits ribosomal protein synthesis and induces G1 prolongation in mitogen–activated T lymphocytes. Immuno, 1995, 155 (7): 3418–3426.
4. Hideki Kawasome, Philip Papst, Saiphone Webb, et al. Targeted disruption of p70s6k defines its role in protein synthesis and rapamycin sensitivity. Proc Natl Acad Sci U S A. 1998, 95 (9): 5033–5038.
5. Romanelli A, Dreisbach VC, Blenis J. Characterization of phosphatidylinositol 3–kinase–dependent phosphorylation of the hydrophobic motif site Thr(389) in P70 S6 kinase 1. J Biol Chem, 2002, 277(43): 40281–40289.

6. Lekmine F, Uddin S, Sassano S et al. Activation of the p70 S6 kinase and phosphorylation of the 4E-BP1 repressor of mRNA translation by type I interferons. J Biol Chem, 2003, 278(30): 27772-27780.
7. Manuel H, Eric K R. The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. Oncogene, 2000, 19: 6680-6686.
8. Thomas G. The S6 kinase signaling pathway in the control of development and growth. Biol Res, 2002, 35:305-313.
9. Liu yi, Tian Yulou, Yu Bingzhi, et al. China Oral Medical Magazine, 2002, 37(2):123-125.
10. Sh:ma H, Pende M, Chen Y, et al. Disruption of the P70 S6K gene reveals a small mouse phenotype and a new functional S6 kinase. EMBO J, 1998, 17:6649-6659.