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ORIGINAL ARTICLE

PRODUCTION OF GROWTH-RELATED ONCOGENE PROTEIN-α IN A HUMAN ORAL SQUAMOUS CELL CARCINOMA CELL LINE STIMULATED WITH TUMOR NECROSIS FACTOR-α: ROLE IN TUMOR ANGIOGENESIS AND TUMOR PROLIFERATION

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Abstract The CXC chemokine growth-related oncogene protein-α (GRO-α) has a wide variety of biological activities including as neutrophil trafficking or migration of vascular endothelial cells. In addition, studies have shown a crosstalk between tumor cells and vascular endothelial cells; GRO-α released by endothelial cells induces invasion of tumor cells toward endothelial cells, indicating an importance of GRO-α in a tumor environment. Oral squamous cells are reported to produce GRO-α in response to cytokines such as tumor necrosis factor-α (TNF-α). However, little is known about how GRO-α is involved in oral cancer. Here, we investigated the biological role of GRO-α for both tumor growth and angiogenesis in oral squamous cell carcinoma cells. We first evaluated the effect of TNF-α on GRO-α expression in three oral cancer cells from different origins. Among the cell lines we used, KOSC-2 cells expressed the highest amount of GRO-α mRNA in response to TNF-α. TNF-α-treated condition medium from KOSC-2 cells enhanced endothelial cell chemotaxis and the chemotactic activity was partially inhibited by the addition of neutralizing anti-GRO-α antibody. In addition, GRO-α exerted tumor cell migration of KOSC-2. From these results, we conclude that GRO-α may contribute to both angiogenesis and proliferation in oral cancer.

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Key words: growth-related oncogene protein-α (GRO-α); tumor necrosis factor-α (TNF-α); oral squamous cell carcinoma cells.

原　著

培養ヒト口腔扁平上皮癌細胞における TNF-α 依存的な GRO-α の誘導：GRO-α による血管新生作用と腫瘍増殖作用について

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抄録　CXC ケモカインファミリーである GRO-α は，好中球走化性因子として知られている他，腫瘍増殖能や血管新生能を有することが明らかとなっている．これまでに口腔粘膜上皮の GRO-α 産生は報告されていたが，GRO-α の口腔癌における役割は不明である．そこで本研究では口腔癌における GRO-α を介した血管新生作用や腫瘍増殖効果について実験的に検討した．３種類の口腔扁平上皮癌由来細胞に TNF-α 処理をしたところ，GRO-α の発現量は細胞間で大きく異なっており，TNF-α 依存的な GRO-α 産生は個々の腫瘍細胞の性質に依存することが示唆された．GRO-α を最も多く産生した KOSC-2(舌癌由来細胞株)の TNF-α 処理後の培養上清は，血管内皮細胞の走化性を亢進し，GRO-α 特異的な中和抗体の添加はその亢進を部分的に抑制した．さらに，ヒト組み換え型 GRO-α は KOSC-2 の増殖を促進した．これらの結果から，口腔癌において GRO-α は腫瘍の増悪因子である可能性が示唆された．

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キーワード：GRO-α；腫瘍壊死因子(TNF-α)；口腔扁平上皮癌．

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Introduction

Tumor necrosis factor-α (TNF-α) regulates a variety of biological functions related to inflammatory reactions, cell growth and apoptosis; and the most important source of TNF-α is macrophages[1]. TNF-α affects carcinoma cells to induce the expressions of many cytokines[2,3]. Constitutive activation of nuclear factor-κB (NF-κB) is observed in many types of cancer cells, strongly suggesting a critical role in cancer development and progression[4]. Among several carcinogens, TNF-α is thought to be the most potent activator of NF-κB[5]. In the tumor mass, tumor-associated macrophage (TAM) should be major source of TNF-α[6].

Growth-related oncogene protein-α (GRO-α) / CXCL1 was first identified as a growth factor of melanoma[7,8]. GRO-α belongs to the C-X-C chemokine family and has chemotactic activity for neutrophils[9]. Some types of the C-X-C chemokine family, which contain the sequence Glu-Leu-Arg (the ELR motif) in front of the C-X-C motif, have been shown to possess a potent angiogenic property[10]. Interleukin-8 (IL-8) / CXCL8, epithelial and neutrophil activating protein-78 (ENA-78) /CXCL5, and GRO-α are the members of this group[10]. A variety of chemokines including GRO-α are rapidly and markedly induced by TNF-α[11]. This indicates that GRO-α acts as the secondary mediator in response to TNF-α. TNF-α has also be reported to induce GRO-α normal oral keratinocytes; however, the role of GRO-α in oral squamous cells has not been proven by experimental analysis[12].

In healthy oral mucosa, IL-8 and monocyte chemotactic protein-1 (MCP-1) /CCL2 mRNA are constitutively expressed whereas mRNA expression of GRO-γ /CXCL3, a member of GRO family chemokine, is significant lower[13]. In contrast, high level of GRO-α expression is shown to be correlated with both tumor angiogenesis and lymph node metastasis in oral cancer[14]. Furthermore, microarray analysis revealed that GRO-α is more markedly expressed in oral cancer cells than in normal oral epithelial cells[15]. These suggest the essential role of GRO-α in oral cancer cells.

Endothelial cells express NF-κB-dependent GRO-α, mostly in response to TNF-α[16]. CXC chemokines including GRO-α and IL-8 secreted by endothelial cells have been shown to induce tumor cell invasion[17]. On the other hand, the role of GRO-α, which is produced from oral squamous carcinoma, is incompletely understood.

We have been studying the effect of TNF-α on human oral squamous cell carcinoma, and here we report the expression of GRO-α is cell line-specific, even in response to TNF-α. We also studied the effect of GRO-α on tumor growth and endothelial cell chemotaxis.

Materials and Methods

Reagents

Cell culture medium Humedia EB-2 and its supplements were purchased from Kurabo (Osaka, Japan). Primer oligo(dT)$_{12\text{-}18}$ and M-Mulv reverse transcriptase were from GIBCO-BRL (Gaithersburg, MD, USA). Digoxigenen (DIG)-labeling and detection systems were obtained from Boehringer Mannheim (Mannheim, Germany) and a GRO-α enzyme-linked immunosorbent assay (ELISA) kit from R&D Systems (Minneapolis, MN, USA). An RNeasy total RNA isolation kit and *Taq* DNA polymerase were from Qiagen (Hilden, Germany). A Northern Max kit and a Lig'nScribe kit were from Ambion (Austin, TX, USA).

Cell culture

A cell line of human oral squamous cell carcinoma, KOSC-2, was a generous gift from

the National Institute of Health Science (Tokyo, Japan)[18]. The other human oral squamous cell carcinoma cell lines, HSC-3 and Ca9-22 were purchased from JCRB Cell Bank (Osaka, Japan). The cells were cultured using RPMI-1640 (KOSC-2) or DMEM (HSC-3 and Ca9-22) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin. The cells were subjected to the stimulation with TNF-α when they reached about 80% confluence.

Human umbilical vein endothelial cells (HUVECs) were purchased from KURABO Tokyo, Japan). The cells were cultured in Humedia EB-2 supplemented with 2% FBS, 10 ng/mL recombinant human (r(h)) epidermal growth factor, 5 ng/mL r(h) basic fibroblast growth factor, 1μg/mL hydrocortisone and 10 μg/mL heparin. CD45+ cells were found in the cultures.

RNA extraction and quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the cells using an RNeasy total RNA isolation kit. Single-strand cDNA was synthesized from 1 μg of total RNA using primer oligo(dT)$_{12\text{-}18}$ and M-Mulv reverse transcriptase. A CFX96 Real-Time PCR System (Bio-Rad) was used for quantitative analyses of GRO-α and 18S rRNA expression. The sequences of the primers were:
GRO-α-F (5'-ATGGCCCCGCGTGCTCTCTCC-3'),
GRO-α-R (5'-GTTGGATTTGTCACTGTTCAG-3'),
18S rRNA-F: 5'-ACTCAACACGGGAAACCTCA-3',
and rRNA-R: 5'-AACCAGACAAATCGCTCCAC-3'.
Amplifications were performed using iQ SYBR Green Supermix (Bio-Rad), according to the manufacturer's specifications. Cycling conditions were as follows: 50°C, 2 min; 95°C, 3 min; 40 cycles of 95°C (15s) + 58°C (30 s) + 72°C (30 s). A melting curve was generated by acquiring fluorescence measurements while slowly heating to 95°C at a rate of 0.1°C per second. Melting curves and quantitative analysis of the data were performed using a CFX manager, as previously reported[19].

ELISA for GRO-α

After the treatment with TNF-α, the KOSC-2 cells were washed twice with RPMI- 1640 and incubated for 2 h in RPMI-1640 containing 0.5% human serum albumin (RPMI-HSA). The medium was collected and subjected to ELISA for GRO-α.

Endothelial cell chemotaxis

Endothelial cell chemotaxis was examined using a 24-well chemotaxis chamber as described previously[20]. Briefly KOSC-2 cells were grown to confluence and stimulated for 4 h with 10 ng/mL TNF-α. Then the medium was replaced with Medium 199 containing 0.5% HSA (M199-HSA), and the cells were conditioned for 2 h. Aliquots (100 μL) of the conditioned medium, M199-HSA containing 1 ng/mL r(h) GRO-α, 10 pg/mL vascular endothelial growth factor (VEGF), or control medium were placed in lower chambers and upper chambers filled with 100 μL of HUVEC suspension ($1x10^5$ cells/mL M199- HSA). When indicated, an anti-GRO-α neutralizing antibody was added to the medium. After incubating for 4 h at 37°C, the membrane from each chamber was fixed with methanol and stained with Giemsa solution. Transmigrated cells in random four low-power fields were counted under a microscope.

Wound assay

Confluent monolayers of KOSC-2 cells were wounded using a scalpel and a rubber policeman as described[21]. Then the cultures were washed with 20 mM phosphate-buffered saline, pH 7.4 (PBS), and further incubated in the conditioned medium of the cells stimulated for 4 h with 10 ng/mL TNF-α. The cells were washed with PBS, fixed with 10% formaldehyde, and photographed under a microscope. Control

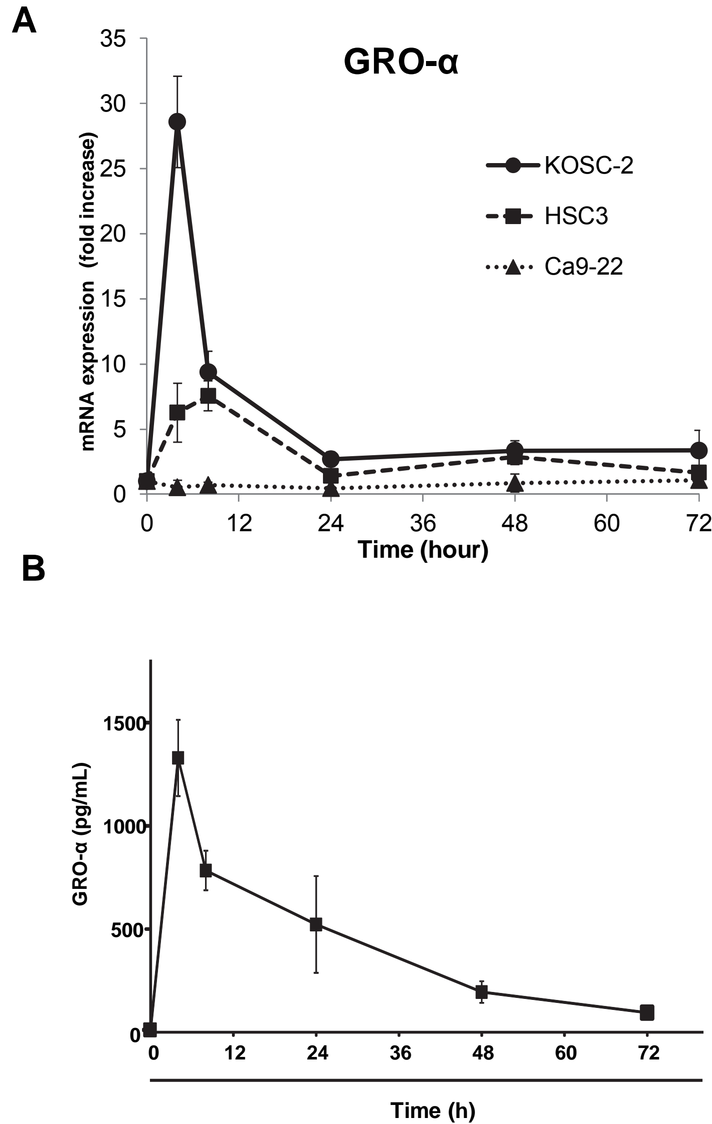

Fig. 1 Time course of the expression of GRO-α in KOSC-2 cells stimulated with TNF-α. (A) KOSC-2 (●), HSC-3 (■), and Ca9-22 (▲) cells were incubated with 1 ng/mL of TNF-α for 4-72 h. mRNA expression of GRO-α or 18s rRNA were analyzed by real-time RT-PCR. (B) KOSC-2 cells were incubated with 1 ng/ml of TNF-α for 4-72 h. The conditioned medium was collected and ELISA was performed. Means (±SD) of three experiments shown.

medium and the medium containing 1 ng/mL r(h)GRO-α were also tested in parallel.

Statistics

For chemotaxis assay (Fig.3.), data were analyzed using one-way analysis of variance (ANOVA) to compare the treatment effects. Tukey's post-hoc analyses were applied for multiple comparisons, with the statistical significance set at $P<0.05$.

Results

Expression of GRO-α in oral squamous cell lines stimulated with TNF-α

We first asked whether most of the oral squamous cancer cells can induce GRO-α in response to TNF-α. In this study, we used three oral cancer cells from different donors to observe GRO-α expression in response to TNF-α. TNF-α (10 ng/mL) transiently expressed

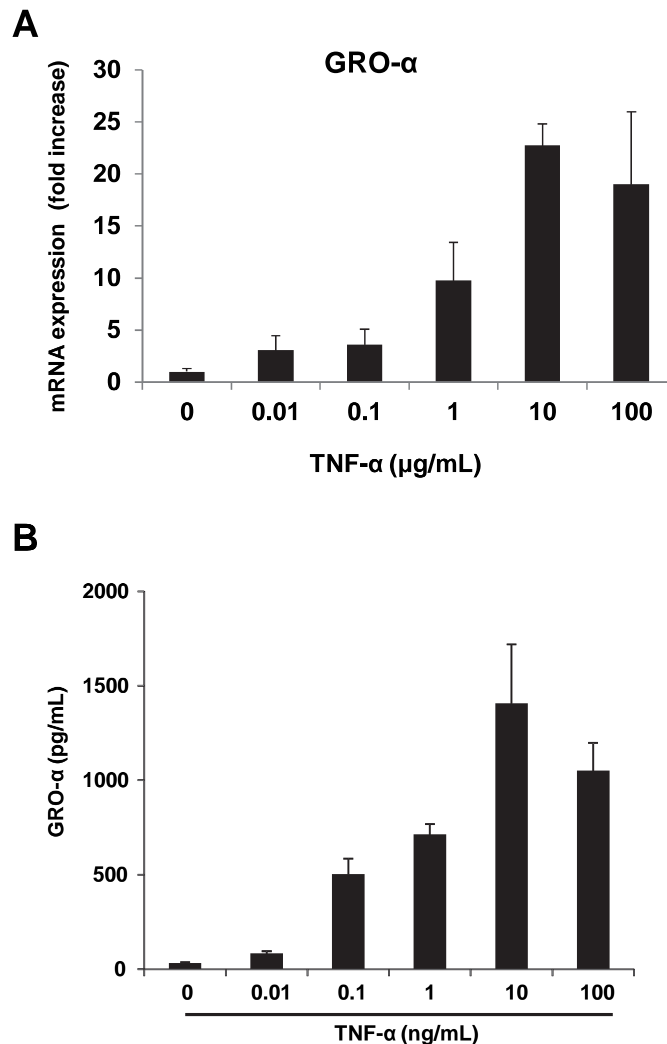

Fig. 2 Concentration-dependent induction of GRO-α by TNF-α. KOSC-2 cells were incubated with 0.01- 100ng/mL TNF-α for 4 h. (A) The expression of mRNA for GRO-α or GAPDH was analyzed by RT-PCR. (B) The conditioned medium of KOSC-2 cells was collected and subjected to ELISA for GRO-α. Means (± SD) of three experiments are shown.

GRO-α in KOSC-2 and HSC-3 cells. In both cells, GRO-α mRNA reached the maximal level 4 h after the stimulation with TNF-α (Fig 1A). The induced levels of mRNA levels of GRO-α in KOSC-2 were markedly higher than that in HSC-3 cells. In contrast, no such increase of GRO-α was observed in Ca9-22 (Fig 1A). These observations suggested that the induction of GRO-α in response to TNF-α varies depending on the cell type. The time course of GRO-α protein secretion corresponded with that of the mRNA expression (Fig. 1B).

TNF-α enhanced GRO-α mRNA expression of KOSC-2 cells in a concentration- dependent manner (Fig. 2A). The expression of GRO-α was observed from the treatment with 0.1 ng/mL TNF-α. TNF-α also stimulated the secretion of GRO-α protein and the maximal effect was observed at 10 ng/mL (Fig. 2B).

GRO-α has chemotactic activity for endothelial cells

The results of endothelial cell chemotaxis are summarized in Fig. 3. VEGF is known as

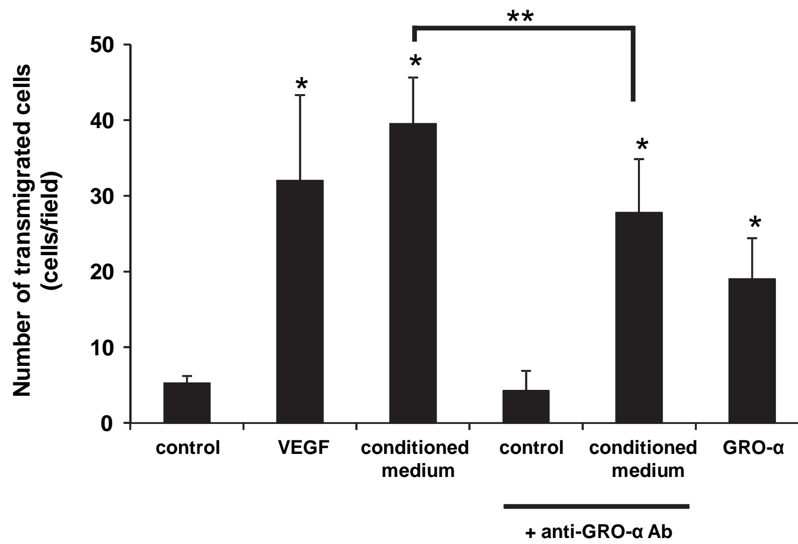

Fig. 3 Endothelial cell transmigration in response to GRO-α. Control medium (M199-HSA), VEGF (100 pg/mL, in M199-HSA). GRO-α (1 ng/mL, in M199-HSA) or conditioned medium (from KOSC-2 stimulated with TNF-α for 4 h) was placed in lower chambers, and upper chambers were filled with HUVEC suspension. When indicated, anti-neutralizing GRO-α antibody was added to the conditioned medium. After incubating for 4 h at 37°C, the membrane was fixed and stained with Giemsa solution. Transmigrated cells in random four fields were counted under a microscope. *$P<0.05$ statistically significant difference compared with the control, **$P<0.05$ vs TNF-α-treated conditioned medium.

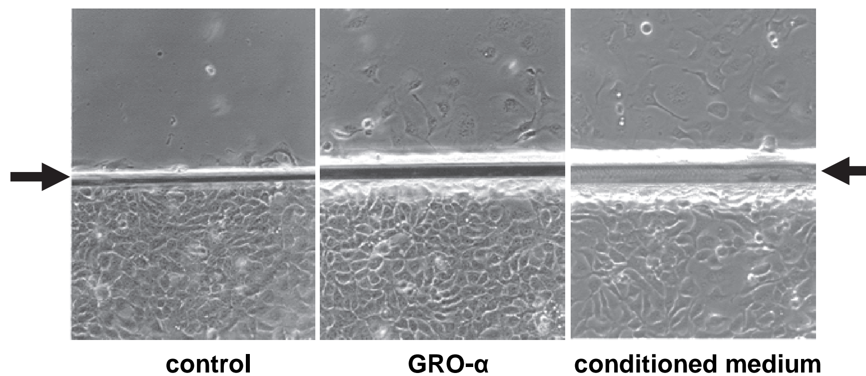

Fig. 4 KOSC-2 cells migration in the presence of GRO-α. Confluent monolayers of KOSC-2 cells were wounded as described in "Materials and Methods". The cells were incubated in the presence or absence of 1 ng/mL GRO-α, or conditioned medium (from KOSC-2 cells stimulated with TNF-α for 4 h) for 20 h, then fixed and photographed.
The arrows point to the original edge of the wound. Data shown represent from two independent experiments.

a potent angiogenic factor, and thus we used VEGF as a positive control for this migration assay for endothelial cells. As we expected, only small amount of VEGF could induce chemotaxis in HUVECs. The conditioned medium from TNF-α-treated KOSC-2 cells significantly enhanced the transmigration of endothelial cells, and r(h) GRO-α was also found to be active in this assay. To evaluate the possible role for GRO-α in the TNF-α-treated conditioned medium, we added anti-neutralizing antibody against GRO-α in the conditioned medium, and

found partial, but significant ($P<0.05$) inhibition by GRO-α neutralization. These data suggest a positive role of GRO-α as a secondary mediator. In agreement with this result, r(h) GRO-α induces chemotaxis of HUVECs.

GRO-α promotes migration of KOSC-2 cells

The results of KOSC-2 cell migration in wound assay are shown in Fig. 4. Twenty hours after wound assay, control KOSC-2 cells grew into the wounded area. In countrast, the growth was faster in the cells incubated with the medium conditioned by TNF-α-treated cell. r(h) GRO-α also showed a migration promoting activity on KOSC-2 cells.

Discussion

TNF-α was first identified as a factor that induces necrosis of tumor cells; however, various functions of this cytokine have been demonstrated thereafter[22, 23]. In some case, it serves as a “tumor growth factor”[24]. TNF-α activates transcriptional factors such as AP-1 and NF-κB, and subsequently induces the expression of various chemokines[25, 26].

In the present study, we initially found that TNF-α induces expression of GRO-α in KOSC-2 cells. GRO-α is known to be expressed in various types of cells including endothelial cells, bronchial epithelium, macrophages and polymorphonuclear neutrophils[27-30]. Previous report has shown the expression of GRO-α by TNF-α in oral keratinocytes[12]. However, Ca9-22 derived from an oral squamous cell carcinoma did not express GRO-α in response to TNF-α. Moreover, super-induction of GRO-α was observed in TNF-α-treated KOSC-2 cells. These results suggested that the level of GRO-α is dependent on individual oral squamous cell carcinoma. GRO-α has a neutrophil chemotactic activity and plays an important role in inflammatory responses, but the ubiquitous nature of its expression suggests that GRO-α is involved in biological events other than leukocyte chemotaxis[9, 10]. In fact some members of C-X-C chemokines that contain ELR motif are demonstrated to act as an angiogenic factor, while the members that lack ELR- motif serve as an angiostatic factor[10]. In the present study, we found that the conditioned medium from TNF-α-treated KOSC-2 cells contained a substantial amount of GRO-α protein and enhanced endothelial cell transmigration. r(h) GRO-α was also found to enhance endothelial migration. Although the medium conditioned by the TNF-α-treated KOSC-2 cells contains many endothelial chemotactic factors, such as IL-8, ENA-78, or VEGF (data not shown), GRO-α may partly account for the activity in the conditioned medium.

GRO-α was originally found as a factor that promotes the growth of melanoma cells, and a subsequent report demonstrated the growth-enhancing effect on other malignant tumors[7,8,31]. We demonstrated, in the wound assay, that GRO-α enhances the growth of KOSC-2 cells; and TNF-α may control the autocrine regulation mechanism of the growth of KOSC-2 cells.

In summary, TNF-α stimulates the secretion of GRO-α by KOSC-2 cells and may control the tumor spread through angiogenesis and growth of the tumor cells.

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References

1) Pennica D, Nedwin G E, Hayflick J S, Seeburg P H, Derynck R, Palladino M A, Kohr W J, et al. Human tumour necrosis factor: precursor structure,

expression and homology to lymphotoxin. Nature 1984;312:724-9.

2) Kuninaka S, Yano T, Yokoyama H, Fukuyama Y, Terazaki Y, Uehara T, Kanematsu T, et al. Direct influences of pro-inflammatory cytokines (IL-1beta, TNF-alpha, IL-6) on the proliferation and cell-surface antigen expression of cancer cells. Cytokine 2000;12:8-11.

3) Nakano Y, Kobayashi W, Sugai S, Kimura H, Yagihashi S. Expression of tumor necrosis factor-alpha and interleukin-6 in oral squamous cell carcinoma. Jpn J Cancer Res 1999;90:858-66.

4) Karin M. Nuclear factor-kappaB in cancer development and progression. Nature 2006;441: 431-6.

5) Aggarwal B B. Nuclear factor-kappaB: the enemy within. Cancer Cell,2004;203-8.

6) Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436-44.

7) Richmond A, Lawson D H, Nixon D W, Chawla R K. Characterization of autostimulatory and transforming growth factors from human melanoma cells. Cancer Res 1985;45:6390-4.

8) Richmond A, Thomas H G. Purification of melanoma growth stimulatory activity. J Cell Physiol 1986;129:375-84.

9) Moser B, Clark-Lewis I, Zwahlen R, Baggiolini M. Neutrophil-activating properties of the melanoma growth-stimulatory activity. J Exp Med 1990;171: 1797-802.

10) Strieter R M, Polverini P J, Kunkel S L, Arenberg D A, Burdick M D, Kasper J, Dzuiba J, et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem 1995;270:27348-57.

11) Matsushima K, Morishita K, Yoshimura T, Lavu S, Kobayashi Y, Lew W, Appella E, et al. Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J Exp Med 1988;167:1883-93.

12) Li J, Thornhill M H. Growth-regulated peptide-alpha (GRO-alpha) production by oral keratinocytes: a comparison with skin keratinocytes. Cytokine 2000;12:1409-13.

13) Zehnder M, Greenspan J S, Greenspan D, Bickel M. Chemokine gene expression in human oral mucosa. Eur J Oral Sci 1999;107:231-5.

14) Shintani S, Ishikawa T, Nonaka T, Li C, Nakashiro K, Wong D T, Hamakawa H. Growth-regulated oncogene-1 expression is associated with angiogenesis and lymph node metastasis in human oral cancer. Oncology 2004;66:316-22.

15) Ye H, Yu T, Temam S, Ziober B L, Wang J, Schwartz J L, Mao L, et al. Transcriptomic dissection of tongue squamous cell carcinoma. BMC Genomics 2008;9:69.

16) Karl E, Warner K, Zeitlin B, Kaneko T, Wurtzel L, Jin T, Chang J, et al. Bcl-2 acts in a proangiogenic signaling pathway through nuclear factor-kappaB and CXC chemokines. Cancer Res 2005;65:5063-9.

17) Warner K A, Miyazawa M, Cordeiro M M, Love W J, Pinsky M S, Neiva K G, Spalding A C, et al. Endothelial cells enhance tumor cell invasion through a crosstalk mediated by CXC chemokine signaling. Neoplasia 2008;10:131-9.

18) Inagaki T, Matsuwari S, Takahashi R, Shimada K, Fujie K, Maeda S. Establishment of human oral-cancer cell lines (KOSC-2 and -3) carrying p53 and c-myc abnormalities by geneticin treatment. Int J Cancer 1994;56:301-8.

19) Matsumiya T, Imaizumi T, Yoshida H, Satoh K, Topham M K, Stafforini D M. The levels of retinoic acid-inducible gene I are regulated by heat shock protein 90-alpha. J Immunol 2009;182: 2717-25.

20) Matsumiya T, Imaizumi T, Fujimoto K, Cui X, Shibata T, Tamo W, Kumagai M, et al. Soluble interleukin-6 receptor alpha inhibits the cytokine-Induced fractalkine/CX3CL1 expression in human vascular endothelial cells in culture. Exp Cell Res 2001;269:35-41.

21) Imaizumi T, Itaya H, Nasu S, Yoshida H, Matsubara Y, Fujimoto K, Matsumiya T, et al. Expression of vascular endothelial growth factor in human umbilical vein endothelial cells stimulated with interleukin-1alpha--an autocrine

regulation of angiogenesis and inflammatory reactions. Thromb Haemost 2000;83:949-55.

22) Carswell EA, Old L J, Kassel R L, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A 1975;72:3666-70.

23) Mannel D N, Echtenacher B. TNF in the inflammatory response. Chem Immunol 2000;74: 141-61.

24) Van Den Berg J M, Weyer S, Weening J J, Roos D, Kuijpers T W. Divergent effects of tumor necrosis factor alpha on apoptosis of human neutrophils. J Leukoc Biol 2001;69:467-73.

25) Imaizumi T, Albertine K H, Jicha D L, Mcintyre T M, Prescott S M, Zimmerman G A. Human endothelial cells synthesize ENA-78: relationship to IL-8 and to signaling of PMN adhesion. Am J Respir Cell Mol Biol 1997;17:181-92.

26) Schnyder-Candrian S, Walz A. Neutrophil-activating protein ENA-78 and IL-8 exhibit different patterns of expression in lipopolysaccharide- and cytokine-stimulated human monocytes. J Immunol, 1997;158:3888-94.

27) Becker S, Quay J, Koren H S, Haskill J S. Constitutive and stimulated MCP-1, GRO alpha, beta, and gamma expression in human airway epithelium and bronchoalveolar macrophages. Am J Physiol 1994;266:L278-86.

28) Gasperini S, Calzetti F, Russo M P, De Gironcoli M, Cassatella M A. Regulation of GRO alpha production in human granulocytes. J Inflamm 1995;45:143-51.

29) Goodman R B, Strieter R M, Frevert C W, Cummings C J, Tekamp-Olson P, Kunkel S L, Walz A, et al. Quantitative comparison of C-X-C chemokines produced by endotoxin-stimulated human alveolar macrophages. Am J Physiol 1998;275:L87-95.

30) Wen D Z, Rowland A, Derynck R. Expression and secretion of gro/MGSA by stimulated human endothelial cells. EMBO J 1989;8:1761-6.

31) Loukinova E, Dong G, Enamorado-Ayalya I, Thomas G R, Chen Z, Schreiber H, Van Waes C. Growth regulated oncogene-alpha expression by murine squamous cell carcinoma promotes tumor growth, metastasis, leukocyte infiltration and angiogenesis by a host CXC receptor-2 dependent mechanism. Oncogene 2000;19:3477-86.