Original Article
Loss of IL-24 expression facilitates invasion in lung adenocarcinoma

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Abstract: Transduction of oncogenic KRAS and EGFR into immortalized human airway cells induced up-regulation of cytokine genes including CCL3, IL1B, and IL-24 in common. We focused on IL-24 in the present study. IL-24, a secreted protein of IL-10 family, is expressed in the immune system and modulates inflammatory reaction. Since the discovery of the function inducing melanoma cell differentiation, much attention has been paid to its anti-tumor effects. We performed expression analysis of IL-24 in lung cancer cell lines with quantitative real time PCR. IL-24 mRNA expression levels were markedly decreased in adenocarcinoma cell lines. In addition, the expression in some of the cancer cells was restored by 5-azacytidine and trichostatin A treatment. A forced expression of IL-24 inhibited migration in A549 and in PC-9 human lung cancer cells, which had lost the IL-24 expression. Immunohistochemical study with semi-quantitative measurement of 143 lung adenocarcinoma cases revealed invasive predominant subtypes had significantly lower scores of IL-24 expression than a lepidic predominant subtype (P = 0.0240). These results suggested IL-24 could play a role in the common oncogenic pathway, intrinsically suppress invasion early and disappear late in lung adenocarcinoma progression.

Keywords: IL-24, invasion, lung adenocarcinoma

Introduction

Lung cancer research has been changed drastically by combining genetic information and morphology in recent years. Histological subtypes of lung adenocarcinoma were well correlated with driver oncogene abnormality such as EGFR and KRAS mutation [1]. Molecular targeting agents have been developed for each cancer types [2, 3]. Even though EGFR and KRAS share MAP kinase cascades [4], EGFR mutated adenocarcinoma and KRAS mutated adenocarcinoma were contrasted and often separately discussed owing to the mutually exclusive relationship [5]. Roles of the genes involved in the common signal pathway to promote lung adenocarcinoma development are still largely unknown.

Interleukin-24 (IL-24) is a secreted cytokine that belongs to the IL-10 gene family. Along with several other IL-10 cytokine family members, it is located on chromosome 1q32-33 in humans and physiologically expressed in the immune system such as thymus, spleen, peripheral blood leukocytes and normal melanocytes to engage in immunomodulatory reaction. Since it was identified as a novel gene that is strongly expressed in human melanoma cells induced to terminally differentiate [6], IL-24 has shown prominent roles in inhibiting tumor growth, invasion, metastasis and angiogenesis in various types of cancer [7].

During the search for common downstream targets modulated by both oncogenic KRAS and EGFR transduction in immortalized human airway cells, we focused on IL-24 in the present study. Moreover, we investigated the potential effects of IL-24 transduction on cell growth and migration activities in both a KRAS mutant cell line and an EGFR mutant lung cancer cell line. To elucidate its role in lung adenocarcinoma progression, we investigated associations between the levels of IL-24 expression and histopathological parameters in surgically resected primary lung cancers.
**Material and methods**

**Cell lines and cell culture**

The cell lines used in this study were provided as follows: An immortalized human airway epithelial cell line (16HBE14o, Simian virus 40 (SV40)-transformed human bronchial epithelial cells) described by Cozens AL et al. [8] was kindly provided by Grunert DC (California Pacific Medical Center Research Institute). A subclone of 16HBE14o cells, described as NHBE-T in this study, was used. Immortalized airway epithelial cell lines (HPL1A and HPL1D, SV40-transformed human small airway epithelial cells) were established by Masuda A et al. [9]. Human lung cancer cell lines (HEK293T, A549, EKVX, H23, H226, H358, H441, H522, H820, H358, H1819, H2087, H460, H1299, HOP62, and HOP92) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Human lung cancer cell line LC2/ad, LCAM1, Lu65, Lu130, Lu135, Lu139, and RERF-LC-KJ were purchased from the Riken Cell Bank (Tsukuba, Japan). Human lung cancer cell lines, PC-3, PC9, and HARA were from Immunobiological Laboratories Co. (Gunma, Japan). Human lung cancer cell lines TKB1, TKB2, TKB4, TKB5, TKB6, TKB7, TKB8, TKB9, TKB12, TKB14, TKB15, TKB17, and TKB20 were obtained from Dr. Hiroshi Kamma via Dr. Takuya Yazawa (Dokkyo Medical University School of Medicine, Tochigi, Japan). Primary normal human bronchial epithelial cells (NHBE) and primary small airway epithelial cells (SAEC) were purchased from Lonza (Tokyo, Japan). Cancer cell lines and immortalized cell lines were cultured in either Roswell Park Memorial Institute (RPMI 1640; Wako, Osaka, Japan) or Dulbecco's modified Eagle's medium (DMEM; Wako) supplemented with 10% fetal bovine serum (FBS; Biowest, Nuaille, France) and penicillin-streptomycin (Gibco-Invitrogen). SAEC and NHBE were cultured in defined keratinocyte serum free medium (Gibco-Invitrogen). All cells were maintained in a humidified incubator with 5% CO₂ at 37°C.

**Plasmid construction**

The pro-retrovirus vectors bearing KRAS [G12 wild-type (G12) and V12 mutant] [10] and EGFR [wild type (WT), Del (deletion of exon 19: E746-A750), L858R (point mutation in exon 21)] [11] were previously described. Complementary DNA (cDNA) coding IL-24 transcript variant 1 (NM_006850.3) was PCR-amplified using cDNA from NHBE cell line and inserted into the pro-retrovirus vector pQCXIP (BD Clontech, Palo Alto, CA, USA). The pro-retrovirus without any cDNA, described as Mock, was used for empty control vector.

**Retroviral-mediated gene transfer**

The pQCXIP-based expression vectors and the pCL10A1 retrovirus-packaging vector (Imgenex, San Diego, CA) were cotransfected into HEK293T cells with Lipofectamine 2000 reagent (Invitrogen). At 24 hours after the transfection, conditioned medium was recovered as a viral solution. Desired genes were introduced by incubating cells with the viral solution containing 10 g/ml of polybrene (Sigma) for 24 hours. Cells stably expressing desired genes were selected with 2.5 g/ml of puromycin (Invitrogen) for 1 to 3 days. The pooled clones were used for biological analyses and expression profiling.

**Microarray analysis**

Gene expression in the empty vector (MOCK), EGFR WT, EGFR Del, EGFR L858R transduced NHBE-T cells was comprehensively evaluated with Agilent Array SurePrint G3 Human Gene Expression 8×60 K v2 (Agilent Technologies). Total RNA was extracted from the cells immediately after completing the selection (5 days post-transduction) using the RNA easy kit (Qiagen). Labeling and hybridization were performed according to the manufacturer's recommendations (Agilent Technologies). Probes showing an absence call were excluded. Then, normalization of signal values was performed with computer software (GeneSpring, Agilent technology, Palo Alto, CA). Probes (genes) were screened, the signal values of which were elevated or reduced more than tenfold by both EGFR Del and L858R transduction in comparison with the empty vector transduction. In addition, genes of the signal values fulfilling all the following requirements were selected. The genes up-regulated by oncogenic EGFR transduction should have signal values of: Mock<WT, WT<Del, and WT<L858R. On the contrary, the ones down-regulated should have signal values of: Mock>WT, WT>Del, and WT>L858R.

**Quantitative reverse transcription-PCR**

First-strand cDNA was synthesized from total RNA of cell lines with the SuperScript First-Strand Synthesis System according to the protocols of the manufacturer (Invitrogen). The cDNA generated was used as a template in
real-time PCR with SYBR Premix EXTaQ (TaKaRa Bio, Otsu, Shiga, Japan) and ran on a Thermal Cycler DICE real-time PCR system (Takara). The primer sets used for the detection of IL-24 were purchased from TAKARA Perfect Real Time support system. The means and standard deviations of the copy number of the gene normalized to the value for GAPDH mRNA were statistically obtained from triplicate reactions.

**Treatment with 5-azacytidine and trichostatin A**

Cells were treated either with 10 mM of 5-azacytidine (Sigma, St. Louis, MO) for 72 hours by exchanging the medium every day or with 300 ng/ml of trichostatin A (Wako, Osaka, Japan) for 24 hours. Cells were also treated with 5-azacytidine for 48 hours and then with a combination of 5-azacytidine and trichostatin A for an additional 24 hours.

**Western blotting**

The cells grown to sub-confluence were solv ed with extraction buffer. After centrifugation, supernatants were recovered as protein extracts. The extracts were mixed with equal volumes of SDS buffer, and then boiled. The samples were subjected to SDS-polyacrylamide gel electrophoresis, and transferred onto nitrocellulose membrane (Amersham). The membranes were incubated with 1.0% non-fat dry milk in 0.01 M/L Tris-buffered saline containing 0.1% Tween-20 to block non-immunospecific protein binding, and then with 0.1 g/ml of primary antibody against either EGFR (Cell Signaling Technology, Beverly, MA) or IL-24 (GenHunter, Nashville, TN) or β-actin (Sigma). After washing with 0.01 M/L Tris-buffered saline containing 0.1% Tween-20, the membranes were incubated with animal-matched horseradish peroxidase-conjugated secondary antibodies (Amersham). Immunoreactivity was visualized with the enhanced chemiluminescence system (ECL, Amersham).

**Cell growth assay**

Cells (2.5×10⁵) were seeded onto a 10-cm culture dish (Iwaki), and grown to a semiconfluent state for 5 to 7 days. The cells were counted, and 2.5×10⁵ cells were seeded again onto a 10-cm dish. Several passages were repeated in the same manner. The sum of population doublings (PDLs) at each point, was calculated by the formula ΣPDLn = log2 (countn/2.5×10⁵) + ΣPDLn-1.

**Colony formation assay**

Cells (2.5×10⁴) were seeded onto a 10-cm culture dish (Iwaki), and grown for an optimal period (8 to 14 days). The cells were fixed with methanol and Giemsa stained, and colonies visible in scanned photographs were counted.

**Cell migration assays**

Trans-well chambers (24 wells, 8 μm pore size, Becton Dickinson, Franklin Lakes, and NJ) were used for the assay. The lower chambers were filled with 0.7 ml of DMEM or RPMI containing 10% FBS, and the cells (5.0×10⁴/0.2 ml of DMEM or RPMI without fetal bovine serum) were seeded onto the filters of the upper chambers. After 24 hours of incubation, non-migrated cells on the upper side of the filter membranes were removed. The cells migrating through were fixed with methanol, and stained with Giemsa (Merck, Darmstadt, Germany). The cells in three fields of 1.0 mm² on filter membranes were counted.

**Primary lung adenocarcinoma**

A total of 143 adenocarcinomas were removed by radical surgical resection at Kanagawa Prefectural Cardiovascular and Respiratory Center (Yokohama, Japan). This study was approved by the ethics committees of Yokohama City University and Kanagawa Prefectural Cardiovascular Respiratory Center Hospital. Informed consent for research use of the resected materials was obtained from all the subjects. Histological types and disease stages were determined according to WHO Classification of Tumours of Lung, Pleura, Thymus & Heart (4th edition) [12] and International TNM Classification System (7th edition of the UICC) [13]. According to the 4th edition of WHO classification, papillary, acinar, solid and mucinous subtypes were gathered as an invasive predominant group. Although a micropapillary subtype should be included in the group, none of our cases had micropapillary “predominant” histology. The rest of the major adenocarcinoma subtype was a lepidic predominant subtype including adenocarcinoma in situ, minimally invasive adenocarcinoma and lepidic predominant invasive adenocarcinoma, which represented a non-invasive predominant group.

**Search for EGFR and KRAS mutations**

The tumorous part was dissected from formalin-fixed, paraffin-embedded tissue sections.
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The sample DNA was extracted using Nucleospin DNA kit (Takara). A fragment of the coding exons of oncogenic EGFR (exon 18, 19, 20 and 21) and of oncogenic KRAS (exons 2 and 3) was PCR-amplified using HotStar-TaqDNA polymerase (Stratagene) according to the manufacturer’s instruction. PCR products were purified with ExoStar (Amersham), and subjected to a dye-terminator reaction using a Big Dye version 3.1 kit (Applied BioSystems, Foster City, CA). Primers used for PCR and DNA sequencing were referred from the previous reports [14, 15].

**Immunohistochemistry**

Tumor sections were cut from formalin-fixed, paraffin-embedded tissue blocks. Contents of the analyzed cases were described in the result table. Sections were deparaffinized, rehydrated, and incubated with 3% hydrogen peroxide, followed by 5% goat serum to block endogenous peroxidase activities and non-immunospecific protein binding. Sections were boiled in citrate buffer (0.01 M, pH 6.0) for 15 minutes to retrieve masked epitopes and then incubated with a primary antibody against IL-24 (GenHunter). IL-24 antibody specificity was confirmed with western blotting. Immunoreactivity was visualized using an Envision Detection System (DAKO), and nuclei were counterstained with hematoxylin. IL-24 immunohistochemical expression levels were described as follows. The faint level was defined as weaker than the level in the bronchioles but not negative. The modest level was defined as a level equivalent to that in the bronchioles. The strong level was defined as an unequivocally stronger level than that in the bronchioles. The strong level was defined as an unequivocally stronger level than that in the bronchioles. The immunohistochemical (IHC) expression score was determined as an average level in the maximal tumor section (if 30%, 10%, 50%, and 10% of neoplastic cells in the maximal section of tumor were negative, faint, modest, and strong levels, respectively, the average level was calculated as “1.4 = 0.3×0+0.1×1+0.5×2+0.1×3”).

**Statistical analysis**

Statistical analyses were conducted using JMP version 9.02 for Windows (SAS, Cary, NC). Error bars in graphical data represent mean ± standard deviation. Statistical significance was determined using Student’s t-test for two variables and one-way ANOVA for three or more variables. All reported P values are two-tailed, and for all analyses, P<0.05 is considered statistically significant.

**Results**

**Downstream molecules whose expressions modulated by the oncogenes**

Downstream molecules of oncogenic KRAS gene were comprehensively searched in the preceding study using microarray analysis, in which the mutant KRAS gene was transduced into immortalized airway cells (NHBE-T cells) [16]. Similarly, those of oncogenic EGFR genes were searched. cDNAs of wild type EGFR (WT), EGFR exon 19 deletion (Del) and EGFR exon 21 L858R point mutation (L858R) were transduced into NHBE-T cells by retroviral-mediated gene transfer. Oncogenic EGFR genes caused growth suppressive effect and cytological atypia (Figure 1). The down-regulated genes and up-regulated genes were listed (Tables 1, 2). These two microarray analyses elucidated that some cytokine genes, CCL3, IL1B, and IL-24, were commonly up-regulated in the two oncogenes-transduced cells. They may be involved in a shared oncogenic pathway of lung adenocarcinoma. We highlighted IL-24 to proceed to further analyses.

**IL-24 mRNA expression in the lung cancer and non-cancer cell lines**

The oncogenic EGFRs and KRAS transduced NHBE-T cells showed higher levels of IL-24 compared to the wild type genes or empty vector transduced NHBE-T cells, which confirmed the microarray analyses (Figure 2A). We also measured IL-24 mRNA levels in lung cancer cell lines, immortalized human airway cell lines, a normal human bronchial cell line and a human small airway cell line. IL-24 mRNA levels were decreased in adenocarcinoma cell lines and small cell carcinoma cell lines compared to other subtypes of lung cancer cell lines and normal human cells (Figure 2B). Notably, the levels were under detection limit in H441, H522, PC-3, TKB20, H460, and TKB12 cells.

**Effects of DNA methyltransferase inhibitor and histone acetyltransferase inhibitor on IL-24 mRNA expression in lung cancer cell lines**

To evaluate the expression regulation of IL-24 in human lung cancer cells, treatment with an inhibitor for a DNA methyl-transferase (5-AZA-dc) in combination with an inhibitor for histone deacetylase (trichostatin A, TSA) was performed. It restored IL-24 expression in A549, H460, RERF-LC-KJ, TKB-5, and H1819 human...
lung cancer cells, whereas non-neoplastic airway epithelial cells (SAEC) demonstrated no restoration of IL-24 expression (Figure 3). These data suggested epigenetic modification
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Table 2. Up-regulated genes

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Genbank</th>
<th>Map</th>
<th>Mock</th>
<th>EGFR WT</th>
<th>EGFR Del</th>
<th>EGFR L858R</th>
</tr>
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<tr>
<td>CCL3</td>
<td>NM_002983</td>
<td>1q12</td>
<td>-5.087 A</td>
<td>-4.322 A</td>
<td>-1.148</td>
<td>P</td>
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<tr>
<td>IL24</td>
<td>NM_001185156</td>
<td>1q32</td>
<td>-6.073 A</td>
<td>-0.359 P</td>
<td>1.490</td>
<td>P</td>
</tr>
<tr>
<td>CSF3</td>
<td>NM_000759</td>
<td>17q11.2-q12</td>
<td>-5.882 A</td>
<td>-2.623 P</td>
<td>-0.041</td>
<td>P</td>
</tr>
<tr>
<td>IL24</td>
<td>NM_001185156</td>
<td>1q32</td>
<td>-1.725 P</td>
<td>2.589</td>
<td>4.423</td>
<td>P</td>
</tr>
<tr>
<td>KRT6A</td>
<td>NM_005554</td>
<td>12q13.13</td>
<td>-5.150 A</td>
<td>-1.736 A</td>
<td>-0.428</td>
<td>P</td>
</tr>
<tr>
<td>SCG5</td>
<td>NM_003020</td>
<td>15q13-q14</td>
<td>-6.068 A</td>
<td>-3.953</td>
<td>-2.269</td>
<td>P</td>
</tr>
<tr>
<td>IL1B</td>
<td>NM_000576</td>
<td>2p14</td>
<td>0.563 P</td>
<td>2.844</td>
<td>5.080</td>
<td>P</td>
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<tr>
<td>KRT6C</td>
<td>NM_173086</td>
<td>12q13.13</td>
<td>-6.433 A</td>
<td>-4.895 A</td>
<td>-2.688</td>
<td>P</td>
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<tr>
<td>RSU1</td>
<td>NM_012425</td>
<td>10p13</td>
<td>-4.759 A</td>
<td>-0.041 P</td>
<td>0.101</td>
<td>P</td>
</tr>
<tr>
<td>KRT6B</td>
<td>NM_005555</td>
<td>12q13.13</td>
<td>-2.795 P</td>
<td>-0.452 P</td>
<td>1.151</td>
<td>P</td>
</tr>
<tr>
<td>IL1A</td>
<td>NM_000575</td>
<td>2q14</td>
<td>-0.105 P</td>
<td>2.164</td>
<td>3.280</td>
<td>P</td>
</tr>
<tr>
<td>MMP1</td>
<td>NM_002421</td>
<td>11q22.3</td>
<td>-0.573 P</td>
<td>1.302</td>
<td>2.801</td>
<td>P</td>
</tr>
<tr>
<td>NLRP3</td>
<td>NM_001243133</td>
<td>1q44</td>
<td>-5.303 A</td>
<td>-4.579 A</td>
<td>-1.933</td>
<td>P</td>
</tr>
<tr>
<td>MMP3</td>
<td>NM_002422</td>
<td>11q22.3</td>
<td>-1.293 P</td>
<td>2.087</td>
<td>3.059</td>
<td>P</td>
</tr>
</tbody>
</table>

MOCK, empty vector-transduced NHBE-T; WT, wild-type EGFR transduced NHBE-T; Del, oncogenic mutant of EGFR exon 19 deletion-transduced NHBE-T; L858R, oncogenic mutant of EGFR L858R point mutation-transduced NHBE-T cells; Symbol, gene name; Map, Chromosomal locus; Genbank, gene bank accession number. Flags indicate whether gene expression is present (P) or absent (A).

might affect IL-24 expression in the human lung cancer cells.

Biological effects of IL-24 on cell lines with KRAS or EGFR mutations

To characterize IL-24 function in lung cancer in vitro, cDNA of IL-24 was introduced into A549 and PC-9 cells using retrovirus transfection. Over expression of IL-24 was confirmed by western blot (Figure 4A, 4E). Neither of the cells showed any change in growth activity evaluated by growth curve analysis and colony formation assay (Figure 4B, 4C, 4F and 4G). Migration assay revealed the number of the IL-24 overexpressed cancer cells infiltrating through the membrane was significantly smaller than that of the control cells (Figure 4D and 4H).

Immunohistochemical expression of IL-24 in primary lung cancers regarding pathological factors

Finally, to elucidate the role of IL-24 expression in lung cancer tissue, immunohistochemistry of IL-24 was performed. The expression was localized in cytoplasm of bronchiole cells and cancer cells (Figure 5A). The expression status was semi-quantitatively scored according to the intensity and the proportion of the positive cells. On the basis of cell line expression profiles of IL-24 and current extensive research with driver oncogenes such as EGFR and KRAS, we targeted adenocarcinoma cases. The associations between the scores with histological types/subtypes, vascular involvement or lymphatic canal involvement, pathological stages, Ki-67 growth activity, and oncogenic mutations, were analyzed (Table 3). On the whole, the immunohistochemical scores of well differentiated carcinomas were higher than those of poorly differentiated carcinomas. In addition, the invasive predominant group (papillary, acinar, solid or mucinous predominant subtypes) had significantly lower immunohistochemical scores than the non-invasive predominant group (lepidic predominant subtype) (Figure 5B). Stage I adenocarcinoma cases showed significantly higher scores than Stage II or more advanced cases. No significant association between the IL-24 levels and oncogenic mutations in adenocarcinomas (Table 3).

Discussion

The aim of the present study is to identify essential factors involved in a common signal transduction promoting carcinogenesis of the lung. We focused on IL-24, through a comprehensive search for downstream targets of the two major driver oncogenes, EGFR and KRAS.
Oncogenic EGFR transduction in NHBE-T cells induced marked growth suppression and cytological atypia. This phenomenon had been observed in our oncogenic KRAS transduction as well [16]. A small subset of mutant EGFR overexpressed immortalized esophageal epithelial cells exhibited proliferative arrest and morphology compatible with senescence in the past report [17]. While one might define it as oncogene-induced senescence [18], we considered the effect in immortalized human airway cells to be an initiating model of oncogenic EGFR/KRAS-mediated carcinogenesis to advance further analyses.

Immunohistochemical expression level of IL-24 was stronger in early stages of lung cancer, while it was weaker in advanced stages. Most of the lung cancer cell lines, which generally derived from advanced cancers, lost its expression. Stronger expression of IL-24 was linked with better postoperative outcome in adenocarcinomas at Stage I (data not shown), which was consistent with the previous clinical report.
These data suggested IL-24 could emerge as a suppressor in the early stage and disappear in the advanced stage of lung cancer. No significant difference in the IL-24 levels was found between adenocarcinomas with \( \text{EGFR} \) mutations and with \( \text{KRAS} \) mutations. The result supports our notion that IL-24 is a common player in the KRAS and EGFR-mediated oncogenic pathway. Also, the levels did not differ between the adenocarcinomas with and without those mutations. The other driver onco-
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In inflammatory state, IL-24 is preferentially expressed by T_{H2} cells where STAT6, GATA-binding protein 3 (GATA3), and JUN are suggested to participate in transcriptional regulation [20-22]. Since STAT6, GATA3 and JUN are known to be in downstream of the EGFR-RAS-ERK pathway, these transcriptional factors seem plausible enough to be engaged in up-regulation of IL-24 likewise in response to the oncogenic stimuli such as mutations of KRAS and EGFR. In fact, IL-24 was activated by RAS signaling [23]. On the other hand, the exact molecular mechanism of altered IL-24 expression in the tumor pathogenesis has been currently unclear. It was revealed that treatment with an inhibitor for a DNA methyl-transferase (5-AZA-dc) in combination with an inhibitor for histone deacetylase (trichostatin A, TSA) restored IL-24 expression in several lung cancer cell lines including A549 cells, but not in non-neoplastic airway epithelial cells. The result coincided with a previous observation, which suggested epigenetic modification could alter IL-24 expression in neoplastic cells [24]. Taken together, it is of great interest to investigate the DNA methylation and acetylation in the IL-24 gene promoter region, especially binding sites for STAT6, GATA3 and JUN.

Invasion of lung cancer involves multiple processes such as angiogenesis, degradation of matrix barriers, disruption of cell-cell and cell-matrix adhesion and inducement of cellular motility [25]. Establishment of the experimental model is challenging. Ectopic adenovirus mediated gene transfer of IL-24

**Figure 4.** The effects of forced expression of IL-24 on cell growth and migration. The empty vector (EMP), and IL-24 transcriptional variant 1 (IL24 cDNA) were transduced into A549 cells (KRAS-mutated cell line) (A-D) and PC9 (EGFR-mutated cell line) (E-H). Cells harvested immediately after the selection were examined for the expression of IL-24 and β-actin by Western blotting (A, E). Cells selected were grown and passed several times. Cumulated population doublings are presented as a line chart (B, F). The surviving cells were harvested and counted, and 2.0×10^{4} cells were re-seeded on a 10 cm dish. After 10 days, cells were methanol-fixed and Giemsa-stained. The means and standard deviations (error bars) of colony counts from triplicate experiments are presented (C, G). The cells that migrated through the pores of the trans-well membranes were stained with hematoxylin; magnification, ×100 (D). (H) The results of migration assays. Three independent assays were performed. The means and standard deviations (error bars) of migrated cells are counted.

genes, such as ALK, RET and ROS, might share the common pathway to induce IL-24 expression.
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inhibited invasion and migration of human lung cancer cells in vitro and in vivo [26]. Recently, this process was accomplished by disrupting the SDF-1/CXCR4 signaling pathway [27]. Since retrovirus mediated gene transfection have the ability to integrate genes into the host genome in a stable fashion, IL-24 integrated in the lung cancer cells seemed to recapture the endogenous inhibitory function. Additionally, we confirmed using human clinical samples, for the first time, reduced IL-24 expression was associated with invasive histological subtypes. Therefore, which part of the invasion processes does IL-24 interfere with might be the next question.

Accumulating studies have found anti-tumor effects of IL-24 in various types of cancer cells, including gliomas, ovarian, breast, liver, pancreatic, gastric, colorectal, renal, and prostate cancer cells [28]. Lung cancer is no exception [29-32]. Restoration of IL-24 in progressed lung cancer might become a treatment option. If so, examining IL-24 status before treatment would allow oncologists to select cases applicable of IL-24 treatment.

In conclusion, our results indicated that IL-24 could be involved in the common oncogenic pathway of lung adenocarcinoma, intrinsically inhibit invasion in early carcinogenesis, and disappear as the cancer progresses in part by epigenetic modification. These findings could enhance the current development of IL-24 treatment strategy for lung cancer.

Acknowledgements

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Disclosure of conflict of interest

None.

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Table 3. Immunohistochemical scores of IL-24 in surgically resected lung cancer specimens

<table>
<thead>
<tr>
<th></th>
<th>Expression score (mean ± SD)</th>
<th>P value*</th>
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</thead>
<tbody>
<tr>
<td>ADC (143)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well (56)</td>
<td>0.432±0.568</td>
<td>0.0744</td>
</tr>
<tr>
<td>Mod (69)</td>
<td>0.235±0.446</td>
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</tr>
<tr>
<td>Por (18)</td>
<td>0.250±0.406</td>
<td></td>
</tr>
<tr>
<td>Subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lepidic (42)</td>
<td>0.460±0.551</td>
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<tr>
<td>Acinar (34)</td>
<td>0.350±0.562</td>
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<tr>
<td>Papillary (35)</td>
<td>0.123±0.256</td>
<td></td>
</tr>
<tr>
<td>Solid (18)</td>
<td>0.250±0.406</td>
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<tr>
<td>Mucinous (14)</td>
<td>0.350±0.630</td>
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<td>Non-invasive predominance or Invasive predominance</td>
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<tr>
<td>Non-invasive (42)</td>
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<tr>
<td>Invasive (101)</td>
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<td>Vascular involvement (143)</td>
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<tr>
<td>Absent (83)</td>
<td>0.364±0.548</td>
<td></td>
</tr>
<tr>
<td>Present (63)</td>
<td>0.245±0.416</td>
<td></td>
</tr>
<tr>
<td>Lymphatic canal involvement (143)</td>
<td></td>
<td>0.0714</td>
</tr>
<tr>
<td>Absent (55)</td>
<td>0.409±0.595</td>
<td></td>
</tr>
<tr>
<td>Present (88)</td>
<td>0.255±0.422</td>
<td></td>
</tr>
<tr>
<td>Stage (143)</td>
<td></td>
<td>0.0252</td>
</tr>
<tr>
<td>1 (111)</td>
<td>0.364±0.540</td>
<td></td>
</tr>
<tr>
<td>2, 3 and 4 (32)</td>
<td>0.141±0.259</td>
<td></td>
</tr>
<tr>
<td>Ki-67 labeling index (143)</td>
<td></td>
<td>0.9685</td>
</tr>
<tr>
<td>Low level (&lt;10%)</td>
<td>0.312±0.533</td>
<td></td>
</tr>
<tr>
<td>High level (≥10%)</td>
<td>0.315±0.482</td>
<td></td>
</tr>
<tr>
<td>Oncogenic mutation/ADC (85)</td>
<td></td>
<td>0.1636</td>
</tr>
<tr>
<td>EGFR (29)</td>
<td>0.524±0.675</td>
<td></td>
</tr>
<tr>
<td>KRAS (12)</td>
<td>0.350±0.392</td>
<td></td>
</tr>
<tr>
<td>None (44)</td>
<td>0.277±0.464</td>
<td></td>
</tr>
</tbody>
</table>

*Student’s t-test for two variables and one-way ANOVA for three or more variables. Abbreviations are ADC, adenocarcinoma; WEL, well differentiated; MOD, moderately differentiated; POR, poorly differentiated carcinomas. “None” stands for cases without KRAS or EGFR mutations.

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References


IL-24 in lung adenocarcinoma


