H1L1, a humanized anti-FGF2 monoclonal antibody with potent anti-tumor activity in A549

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Abstract

Fibroblast growth factor 2 (FGF2) and fibroblast growth factor receptor 1 (FGFR1) is up-regulated in many tumors, plays a pivotal role in tumor growth and angiogenesis. Therefore, blocking the interaction of FGF2 and FGFRs is a potential strategy to specific therapeutic target in this disease. Here, we evaluated a H1L1, which a humanized monoclonal antibody against FGF2, anti-tumor activity in A549. In this study, H1L1 has a correctly assembled form. And the purity of H1L1 was reached 98.64%. In addition, H1L1 has the ability to bind with FGF2, and effectively inhibited the proliferation and migration of A549 cells. Taken together, H1L1 has significant anti-tumor effects on A549. The results suggested a rationale for clinical development of H1L1 in lung cancer.

Keywords

Fibroblast growth factor 2 (FGF2), Monoclonal antibody, Lung cancer.

1. Introduction

Fibroblast growth factor 2 (FGF2) is a member of the FGF family, was produced by normal and tumor cells, an extremely potent pro-angiogenic growth factor and plays an important role during embryonic development, tissue homeostasis and metabolism[1-3]. However, abundant evidence shows that FGF2 was aberrantly activated which was concerned with the pathogenesis of many types of cancer[4]. FGF2 exerts its biological activity by interacting with FGFR1. The binding of FGF2 and FGFRs could induce a conformational change and dimerization of the FGFRs[5]. and subsequently results in activation of its intracellular tyrosine kinase domain downstream signaling pathways, including many physiological roles, such as proliferation, differentiation, survival and angiogenesis [6].

Angiogenesis plays a critical step in tumor growth, progression and metastasis. Therefore, targeting angiogenesis have been extensively researched on global. FGF2 and VEGF with potent activity of angiogenesis among many angiogenic factors[7-9]. Monoclonal antibody was designed to suppressed tumor growth and angiogenesis. For instance, thirteen years has passed since the Bevacizumab was successful in clinical trails and approved by the FDA of America for the treatment of different cancers. However, many researches shows that the patients have developed resistance to Bevacizumab when they treated for a long time[7]. Many evidences suggests that the mechanism of this phenomenon was the compensatory up-regulation of platelet derived growth factor, epidermal growth factor and FGF2, which increased activity of FGFR, PDGFR and EGFR pathway[10]. In addition, other researches showed that high levels of serum FGF2 have been correlated with a worse survival for refractory cancer patients[11-13]. Therefore, the rationale of blocking the FGF2/FGFR1 pathway in these setting is to reduce or completely block the signal between FGF2 and tumor cells. On the basis of the evidence for FGF2/FGFR dysregulation in tumor, Several FGF2/FGFR inhibitors were developed, which including gene expression inhibitors; ligand binding inhibitors; ligand trap; tyrosine kinanse inhibitors and anti-FGF2/FGFRs antibodies, have shown promising anticancer and antiangiogenic efficacy in several in vitro assays and in vivo preclinical animal models[4]. Nowadays, the

monoclonal antibody drugs which including anti-FGFR3(NCTO1363024) and anti-FGFR2 (NCT 01881217) are ongoing clinical studied in patients with solid tumors and multiple myeloma[14, 15].

In this paper, we evaluated a novel humanized anti-FGF2 H1L1and by indirect ELISA the H1L1 exhibited specific binding to FGF2. In addition, H1L1 showed strongly anti-tumor activity in vitro. In the future, we will investigate the anti-tumor activity in xenograft models.

2. Materials and methods

2.1 Materials

A549 cells were cultured in DMEM(Gibco) media supplemented with 10% fetal bovine serum(Gibco) and 1% penicillin/streptomycin on plastic at 37℃ in a 95% humidity atmosphere containing 5% CO2.

2.2 Methods

2.2.1 Identification and purity of H1L1

The humanized monoclonal antibody H1L1 was purified by Protein G from the Culture suspension. The Molecular weight of the H1L1 was analyzed by SDS-PAGE, and the purity of the H1L1 was evaluated by SEC-HPLC.

2.2.2 Enzyme immunoassay(ELISA)

The 96-well plates were coated with FGF2 40 ng/well at 4℃ overnight and blocked with 5% non-fat milk. The purified H1L1 against FGF2 were added in different concentration and incubated for 1 h at 37℃. The HRP-conjugated goat anti-human IgG was used to detect the antigen-antibody binding which was incubated for 45 min at 37℃. The plates were stained with DAB and the optical density(OD) was measured at 450 nm in an ELISA reader (BioTek, Highland Park, Winooski, VT, USA)[7].

2.2.3 Cell proliferation assay

The effects of H1L1 on cell viability were evaluated by CCK8 assay. A549 cells (3 x 10^4 cells/100uL/well) were seeded into 96-well plates and cultured with DMED supplemented with 10% FBS and 1% penicillin/streptomycin overnight at 37℃. After treating with DMEM for 12 h, A549 cells were treated with serially diluted H1L1 plus 20 ng/mL FGF2 for 72 h. Thereafter, A549 cells was incubated with Cell Counting Kit-8 solution at 37℃ for 1-3 h (CCK-8; Dojindo Labo-ratories, Kumamoto, Japan) and the optical density(OD) was measured at 450 nm in an ELISA reader. The percentage of alive cells were calculated by comparison with the isotype IgG.

2.2.4 Transwell assay

The migration inhibitory effect of the H1L1 on A549 cells were assessed in transwell chambers (BD Biosciences, Bedford, MA, USA). The A549 cells (1 x 10^5 cells/100μL/insert in serum-free medium containing 20ng/mL FGF2) were transferred to insert of the transwell. The H1L1 (200 μg/mL) was added to the inserts and incubated at 37℃ for 18 h. The lower chambers were DMEM with 10% FBS to act as a chemoattractant. After 18 h, The cells on the upper side of the filters were mechanically removed, and those migrated into the lower side were fixed with 4% paraformaldehyde, stained by 0.1% crystal violet (Meryer, Shanghai, China) and imaged with a computerized imaging system[7]. The percentage of migrated cells were calculated by comparison with the medium group.

2.2.5 Statistical analysis

Statistical differences between different groups were performed with GraphPad Prism 5(GraphPad Software, La Jolla, CA, USA), and student's t-test was used for comparision of two groups, $P<0.05$ were considered statistically significant. assays were performed at least three times independently.

3. Results

3.1 The characteristics of H1L1

H1L1 was purified by using protein G chromatography. The monoclonal antibody has a correctly assembly form that was evaluated by reducing and non-reducing SDS-PAGE analyses. Under reducing conditions, the homodimeric H1L1 band was separated as heavy chain and light chain that migrated as 50 KDa and 25 KDa, respectively (Fig. 1A, 2, 3 lane). Moreover, the purity of the mAb H1L1 assessed by SEC-HPLC was 98.64% (Fig. 1B). These results showed that H1L1 has a good purity.

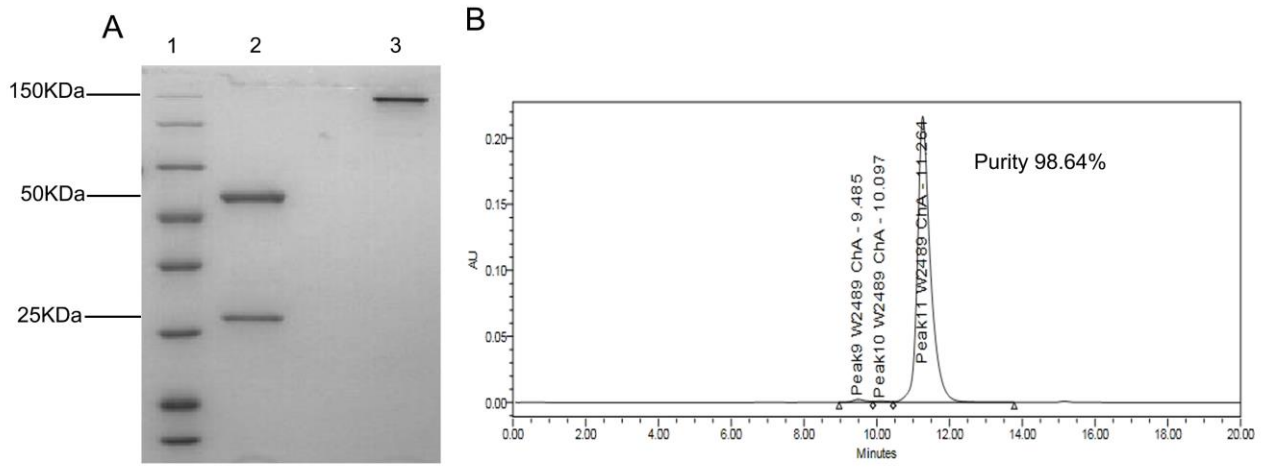

Fig 1. Analysis of the mAb H1L1 (A) SDS-PAGE of purified monoclonal antibody. Lane 1 was loaded with protein ladder; Lane 2 was loaded with reducing mAb H1L1; Lane 3 was loaded with non-reducing mAb H1L1. The samples were separated by using 12% gradient PAGE gels and stained with Coomassie Blue. (B)Analysis of purified mAb H1L1 at 1mg/ml by SEC-HPLC.

3.2 Antigen-binding activity of the H1L1 with FGF2

In order to evaluate whether H1L1 could bind specifically to FGF2, indirect ELISA was performed. The results indicated that the humanized monoclonal antibody H1L1 could bind with FGF2 (Fig. 2).

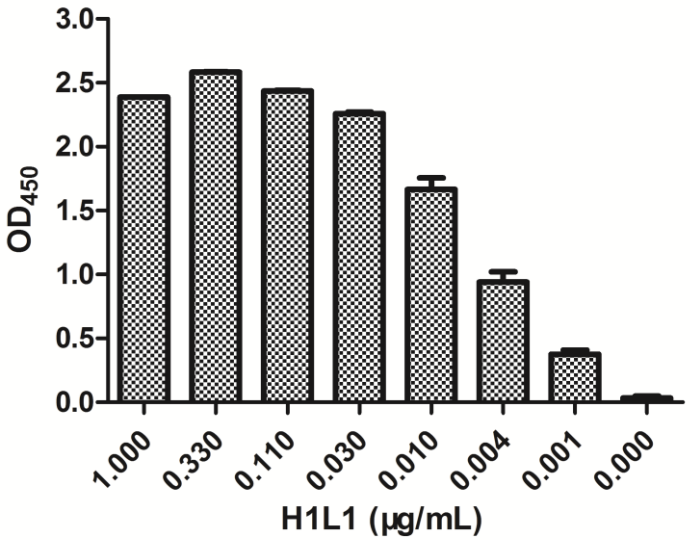

Fig 2. Binding activity of mAb H1L1 to FGF2. Date are from the mean ± SD of three independent experiments.

3.3 The FGF2 activated cell proliferation of A549 cells was remarkably by suppressed by H1L1 in a dose dependent manner

When the concentration of H1L1 was 1000μg/mL, the inhibition rate was 46.41%, (Fig. 3). The results indicated that H1L1 could effectively inhibit the proliferation of A549 cells in a dose dependent manner.

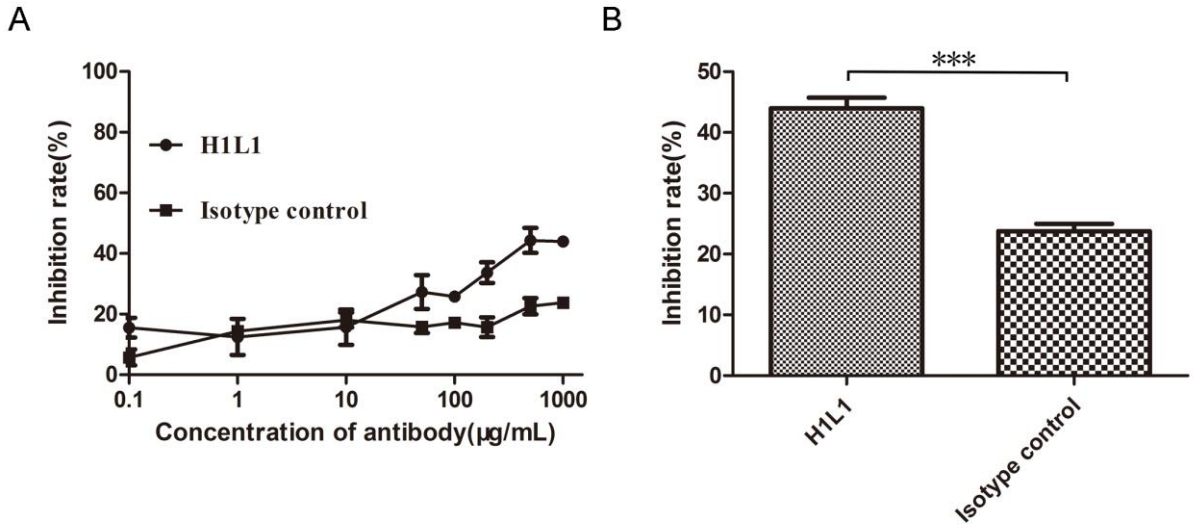

Fig 3. Proliferation inhibition effects of the H1L1 on A549 cells. The A549 cells were transferred to 96-well plates. And cultured with serum-starved with 20 ng/mL FGF2 plus H1L1 at the indicated concentrations and incubated for 72 h. The cell proliferation was assessed by CCK8-kit. Date are from the mean ± SD of three independent experiments. ***p<0.001.

3.4 Transwell assay to analyze migration of A549 cells

The trans well assays were performed to evaluate the migration inhibitory effect of the H1L1 on A549 in different groups. The migrated A549 cells in the H1L1 group were significantly reduced compared to the Medium control (Fig. 4). The cells migration rate of H1L1 and Medium was 36.52% and 86.52%, respectively.

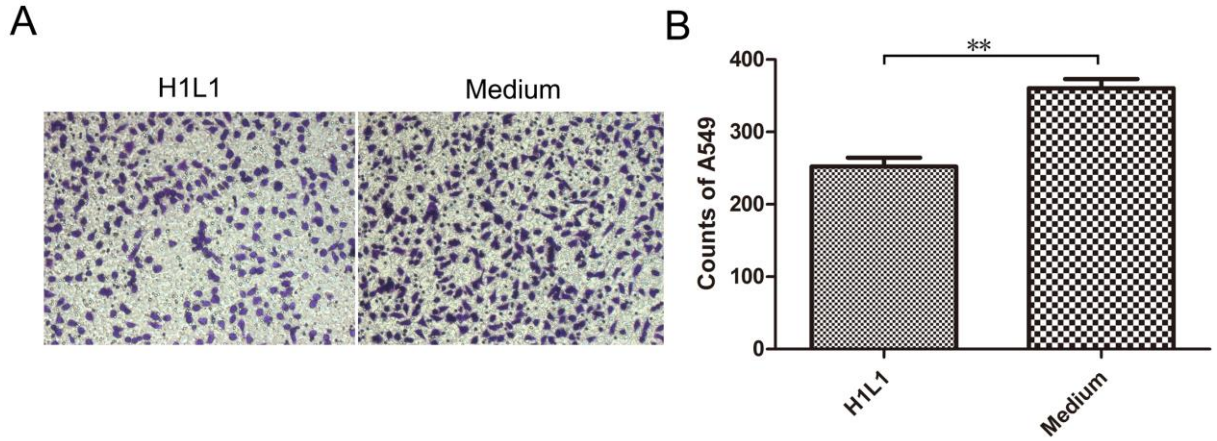

Fig 4. The migration inhibitory effect of H1L1. The A549 cells were seeded into 96-well plates and then treated with different groups (A) A549 were treated with H1L1 and Medium. (B) The quantitative analysis of migrated cells. Date are from the mean ± SD of three independent experiments. **p<0.01.

4. Discussion

FGF2 plays an critical role in the growth, progression, metastasis and angiogenesis in tumors, including lung cancer. These features makes it an interesting target to be explored by anti-tumor

approaches[16]. In previous study, we produced H1L1, a humanized full-length monoclonal antibody targeting FGF2. Here, we evaluated the activity of anti-tumor in lung cancer. The characteristics of H1L1 was cored with IgG1, and the purity was reached 98.64%. H1L1 could specifically bind to FGF2 and neutralize its activity. In addition, H1L1 effectively suppressed migration and proliferation of A549. These results strongly suggested the potential use of this monoclonal antibody to block the FGF2 activited FGFRs signaling in lung cancer.

Six major classes of agents have been developed targeting FGF2, which including ligand binding inhibitors; ligand trap; anti-FGF2/FGFR monoclonal antibodies; tyrosine kinase inhibitors; FGF2 downregulators; gene expression inhibitors[4]. Among these agents, anti-FGF2/FGFR monoclonal antibodies have several potential advantages. Firstly, anti-FGF2 mAb could specific binding with FGF2, which could significantly reduce the adverse reactions, and extended the half-life of the drug, and allowed less frequent of drug administration; Secondly, the potential for direct tumor cell cytotoxicity via antibody-dependent cell-mediated cytotoxicity mechanisms[17]; Thirdly, anti-FGF2 mAbs including H1L1 could bind to the extracellular domain of FGFR and blocks receptor internalization, ultimately leading to shutdown of FGFR signaling; Finally, contrary to murine monoclonal antibody, H1L1 can reduce HAMA[18].

The overexpression of FGF2 and FGFR, the key mediator of angiogenesis, promotes resistance to VEGF inhibition[8, 19, 20]. In a previous study, anti-FGF2 effectively inhibited growth and microvessel density of tumor[7], suggesting that at least part of the anti-tumor effect of anti-FGF2 is mediated by inhibition of tumor angiogenesis. Furthermore, more and more evidence shows that FGF2/FGFR pathway up-regulation acts as a compensatory mechanism of escape from VEGF blockade settings. In these regards, the ability of H1L1 to block FGF2-induced FGFR pathway in our study may contribute to the enhanced anti-tumor effects.

In summary, H1L1 is a humanized monoclonal antibody targeting FGF2 that showed a promising anti-tumor activity in A549 cell in vitro. Our data provide a rationale for future clinical investigations of therapeutic efficacy of H1L1 in lung cancer patients.

Acknowledgements

This research was supported by the Science and Technology Planning Project of Guangdong Province(2013A022100031)

Disclosure Statement

The authors have no conflict of interest to declare.

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