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Effects of small interfering RNAs targeting MAPK1 on gene expression profile in HeLa cells as revealed by microarray analysis

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Abstract

The mitogen activated protein kinases (MAPK) signaling cascade plays an important role in cell life. We proved that small interfering RNAs targeting MAPK1 (siRNA-2) could inhibit HeLa cell growth, but the effects of siRNA-2 on gene expression profile were unclear. Using Affymetrix GeneChip HG-U133A 2.0, we identified the long-term changes for 48 h in gene expression profile in HeLa cell treated by siRNA-2. The results showed that expressions of 181 genes were altered by siRNA-2 and were divided into two groups: (i) one group showed downregulation by siRNA-2, including the proliferation associated genes, small G protein, cytoskeleton associated protein and extracellular matrix associated protein; and (ii) the other group showed upregulation by siRNA-2, including interferon response genes, OAS family, TRIM family and apoptosis associated genes. The results of Real-time quantitative PCR for MAPK1, NUP188, P38, STAT1, STAT2, MPL and OAS1 were consistent with that of gene chip. Two networks were found to react substantially to the downregulation of MAPK1 by siRNA-2. One of the networks regulates cell growth through cell-cycle control, apoptosis and cytoskeleton. The other network is related to interferon-like response. Our findings suggest that siRNA-mediated downregulation of MAPK1 could be an attractive strategy for cancer therapy.

Keywords: siRNA; MAPK 1; Apoptosis; HeLa cell; Microarray

1. Introduction

The mitogen activated protein kinases (MAPK) signalling cascade are membrane-to-nucleus signaling modules and are involved in multiple physiological processes, and consist of three protein kinases: an MEK kinase (MEKK), a dual-specificity MAP kinase kinase (MAPKK or MEK) and an MAP kinase (MAPK) (Seger and Krebs, 1995). There are four major groups of MAPKs in mammalian cells: extracellular signal regulated kinase (ERK), c-jun NH2-terminal kinase/stress-activated protein kinase (JNK/SAPK), p38, and extracellular signal regulated kinase-5 [ERK5, also called Big MAP kinase-1 (BMK1)] (Boulton et al., 1991; Derijard et al., 1994; Han et al., 1994; Kyriakis and Avruch, 2001). ERK is mainly activated by mitogenic stimuli such as growth factors and hormones to induce cell proliferation. In most cell types, the mitogenic signal is relayed from the cytoplasm into the nucleus by nuclear translocation of the ubiquitously expressed p42/p44 isoforms, resulting in activation of a range of transcription factors such as Elk-1. In contrast to MAPKs p42/p44, JNK and p38 are predominantly activated by stress stimuli and inflammatory cytokines and are involved in the phosphorylation of the small heat shock protein Hsp27, in

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the increased cytokine expression and programmed cell death. ERK5 is activated by both stress stimuli and growth factors. These MAP kinases are activated by a dual phosphorylation on Thr–X–Tyr motif, where T and Y are threonine and tyrosine, and X is glutamate, proline or glycine, respectively, in ERK, JNK or p38 (Robbins et al., 1993; Ahn et al., 1991; Payne et al., 1991). Among these signalling cascades, the RAS-mitogen activated protein kinase (MAPK) signalling pathway has long been viewed as an attractive pathway for anticancer therapies, based on its central role in regulating the growth and survival of cells from a broad spectrum of human tumours (Sebolt-Leopold and Herrera, 2004). ERK MAPKs pathway, which contains some proto-oncogenes and several factors, has been already examined in various human cancers. For example, gene amplification of epidermal growth factor receptor (EGF-R) has been reported in glioblastomas, esophageal cancer, lung cancer, ovarian cancer, and breast cancer (van der Valk et al., 1997; D'Amico and Harpole, 2000; Imaizumi et al., 1997; Scoccia et al., 1998; deFazio et al., 2000). HER2-Neu receptor, c-erbB-2, is also overexpressed in breast, ovary, endometrial, and pancreatic cancer (Tuziak et al., 2001; Hellstrom et al., 2001; Rolitsky et al., 1999; Apple et al., 1999). Activated ras mutation has been found in more than 20% of all cancer such as ovarian tumors, colorectal adenoma, pancreatic cancer (Mayr et al., 2006; Hiraoka et al., 2006). The overexpression and activation of ERK MAPKs have also been reported in many cancers such as hepatocell carcinomas, renal cell carcinomas, gastric adenocarcinoma, prostate neoplasm, and breast cancer (Specht et al., 2001; Douziech et al., 1999; Chen et al., 2004, 2007). Since activation of ERK MAPKs induces cell proliferation and transformation in NIH3T3 and MEK inhibitor inhibits cell growth of mouse colonic cancer, ERK MAPKs pathway plays a crucial role for cancer proliferation. MAPKs p42/p44 are ideal targets for anticancer drugs due to their effect inducing proliferation in cells. Inhibition of MEK/ERK activity with inhibitors of ERK activation is sufficient to induce apoptotic cell death and to attenuate invasiveness of tumors (Schmidt et al., 2003; Simon et al., 1999). We proved previously that the downregulation of MAPK 1 by siRNA could inhibit HeLa cell growth (Huang et al., 2006), but the effects of small interfering RNAs targeting MAPK p42 on gene expression profile were unclear. In the present study, we demonstrated that siRNAs targeting MAPK 1 induced apoptosis via two networks: one that regulates cell growth through cell-cycle control, apoptosis and cytoskeleton; the other that is related to interferon-like response. Our findings suggest that the HeLa cell apoptosis induced by MAPK p42 siRNA are related directly and/or indirectly to these cellular machineries.

2. Materials and methods

2.1. siRNA synthesis

In our study, siRNAs were designed using RNAi target finder (http://www.ambion.com/techlib/misc/siRNA_finder.html). The sequences of siRNAs were as follows: siRNA-1 (negative control) sense (CUCUACGUAAGAUCCAGCUUU) and antisense (AGCUGGAUCUUACGUAGAGUU), bearing no homology with any relevant human genes; siRNA-2 sense (GUACUGCACAUAACGCUUCUU) and antisense (GAAGCGUUAUGUGCAGUACUU). And siRNAs were synthesized and purified by means of Silencer™ siRNA Construction Kit (Ambion, Inc., USA).

2.2. Cell culture and transfection

Human cervical cancer cell line HeLa was obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Human HeLa cells (5.0×10^4 cells/ml) were cultured in RPMI1640 supplemented with 10% (v/v) fetal bovine serum, containing 2.0 mmol/L of glutamine and 20 μg of penicillin-streptomycin/ml in 5% CO2, at 37 °C, and were allowed to adhere for 24 h. siRNAs were transfected into HeLa cells by using Lipofectamine™ 2000 (Invitrogen, USA) at the final concentration of 50 nmol/L. Two days after transfection, cells were analyzed for Western blot and apoptosis. All tests were carried out in triplicate.

2.3. MTT assay for cell viability

Cells (1.0×10^4 cells/well) were cultured into 96-well plates. After 24 h, cells were incubated with siRNAs for indicated times at 37 °C in 5% CO2. Afterwards, 20 μL/well of MTT solution (5 mg/mL) was added and incubated for another 4 h. Then supermatants were removed and formazan crystals were solubilized in 200 μL of dimethylsulfoxide. Finally, optical density was determined at 490 nm by an POLARstar$^+$OPTIMA (BMG Labtechnologies, Germany).

2.4. Measurement of HeLa cell apoptosis by Annexin-V/PI staining

HeLa cells were treated with 50 nmol/L siRNAs at 37 °C for 48 h, then harvested and washed twice with PBS. The cells were labeled by incubation with 10 μL of FITC – Annexin V and 5 μL of PI at 250 μg/ml for 10 min in dark at room temperature. Afterward, cells were washed with PBS again and were examined using flow cytometry (BD, USA). Quantification of apoptosis was routinely determined by counting the number of cells stained by FITC-labeled Annexin V.

2.5. Immunoblotting

Cells were plated at 5×10^4 cells/well in six-well plates with RPMI-1640. After being exposed to siRNAs at 50 nmol/L, the cells were washed with PBS and subsequently lysed in 200 μL of lysis buffer (150 mM NaCl, 50 mM Tris–HCl (pH 8.0), 0.01% (w/v) sodium azide, 1% (v/v) NP-40, 1 mM phenylmethylsulfonyl fluoride). Insoluble material was removed by microcentrifugation at 13,000 rpm for 15 min at 4 °C. Cell lysates (80 μg of protein/lane) were subjected to electrophoresis using 10% SDS polyacrylamide gels(SDS-PAGE).