Biomedicine & Pharmacotherapy 78 (2016) 335–341

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Mutation R273H confers p53 a stimulating effect on the IGF-1R-AKT pathway via miR-30a suppression in breast cancer

Fangdong Guo[a,*], Hongshen Chen[a], Jian Chang[b], Lin Zhang[b]

[a] Department of breast and thyroid surgery, The Affiliated Zhongshan Hospital of Dalian University, Dalian 116001, China
[b] The Institute of Cancer Stem Cell, Dalian Medical University, Dalian 116044, China

ARTICLE INFO

Article history:
Received 13 August 2015
Received in revised form 23 January 2016
Accepted 25 January 2016

Keywords:
p53R273H
miR-30a
IGF-1R
AKT

ABSTRACT

p53 is the most highly mutated tumor suppressor in human malignancies. A wide array of p53 mutations has been revealed to play pivotal roles during cancer progression, which abolish anti-tumor functions of wild type p53 but also elicit tumorigenic effects by activating a diverse subset of downstream molecules. R273H mutation of p53 has been closely implicated in human cancer. Here we report miR-30a as a novel downstream target of p53 R273H mutant, which binds to the promoter region to repress miR-30a expression. Consequently, p53 R273H mutant enhances the migratory capabilities of tumor cells that are compromised by exogenous miR-30a over-expression. Our further investigation indicates that p53 R273H mutation unleashes the inhibition effect of miR-30a on IGF-1R expression, thus leading to elevated activation of IGF-1R-AKT signaling cascade in tumor cells.

1. Introduction

Accumulating cancer genomics data present evidence that the tumor suppressor p53 encoding gene *TP53* represents the most highly mutated gene in human neoplasm. A mutation landscape study across 12 major cancer types with The Cancer Genome Atlas (TCGA) sequencing data estimates a pan-cancer mutation rate of 42% for *TP53*, which is more than two fold that of the second most mutated gene *PIK3CA* (at 17.8%) [1]. Ovarian serous carcinoma represents the cancer type with most frequently mutated *TP53*, in which more than 94% samples were identified to carry mutation in this tumor suppressor [2]. Such high mutation rate undoubtedly indicates great significance of p53 mutations in human cancers, which have been proposed to eliminate the functionalities of wild type p53 including cell cycle arrest and apoptosis induction, as well as to contribute to tumorigenesis through modulating a number of downstream signaling units [3–5]. Mutations occur throughout the whole p53 protein, with more populates its DNA binding domain. Among many mutations in this domain, arginine 273 appears to be one of the most highly mutated residues. In this study, we focus on one frequent mutation at this position in p53, R273H that harbors histidine instead of arginine [5,6].

microRNAs (miRNAs) are small non-coding RNAs. In their mature forms, miRNAs normally comprise 19 to 23 nucleotides. Many functions of miRNAs have been demonstrated, with a predominant role in the regulation of protein expression through inhibition of translation or mediating mRNA degradation [7,8]. It is well established that miRNAs are closely implicated in human malignancies. In this report, we illustrate that miR-30a is a direct downstream target of p53 R273H, which represses its expression via binding to the promoter region. As a result, this suppression releases the inhibition of miR-30a on the expression levels of Insulin-like Growth Factor 1 Receptor (IGF-1R), which ultimately leads to increased phosphorylation of its downstream signaling molecules exemplified by AKT and enhanced migration of tumor cells.

2. Materials and methods

2.1. Cell culture and reagents

Human lung cancer cell line H1299 and human breast cancer cell line MDA-MB-468 were obtained from the American Type Culture Collection (ATCC) and cultured with PRMI-1640 medium containing 10% fetal bovine serum (FBS; Gibco-BRL). Medium was renewed every one day and cells were passages before reaching confluence. The following antibodies were used in this study: antibody against AKT (Cell Signaling, USA; 2920s); GAPDH (Santa Cruz Biotechnology, Dallas, TX, USA; SC-25778); p53 (Santa Cruz

* Corresponding author.
E-mail address: fangdongguo2015@163.com (F. Guo).

http://dx.doi.org/10.1016/j.biopha.2016.01.031
0753-3322/

Biotechnology, Dallas, TX, USA; SC-126); phospho-AKT (ser473) (Cell Signaling, USA, 4691s); IGF1R (Cell Signaling, USA; 14534s).

2.2. RNA interference

RNA interference was performed as previously described. Sequence targeting p53 was 5-CGGCGCACAGAGGAAGAGAAT-3, miR-30a mimics and miR-30a inhibitors were purchased from Genepharma Company (Beijing, China).

2.3. Real-time RT-PCR

Total RNA was isolated using Trizol (Sangon Company, Shanghai China). One microgram of total RNA was used to synthesize cDNA using PrimeScriptTM RT reagent kit (Takara, RR047A) according to the manufacturer's instruction pri-miR-30a was amplified with the following primers: pri-miR-30a F:5-ACTTTACAGAATCGTTGC-3; pri-miR-30a R 5-GCAGCTGCAAACATCCGA-3. The primers for mature miR-30a were purchased from Life Technologies.

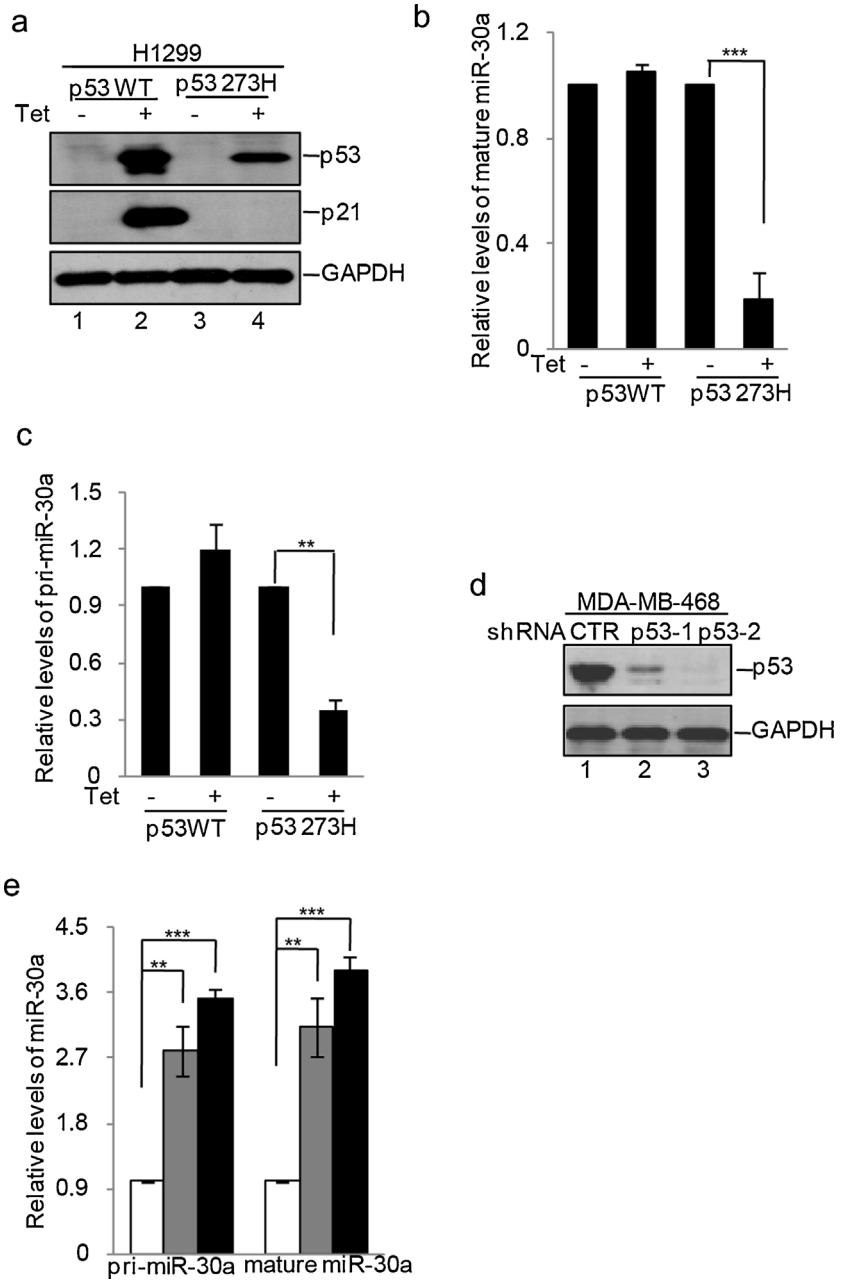

Fig. 1. miR-30a is transcriptional downregulated by p53 R273H mutant. H1299 cells with doxycycline-inducible expression of either wide type p53 or mutant p53 were incubatedwith doxcycline for 24 h. Cell lysates were subjected to western blot analysis with the indicated antibody. (b–c) The expression levels of mature miR-30a and primary miR-30a were examined by q-RT-PCR. The data were obtained from three times independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs Ctr. (d) MDA-MB-468 cells were stable knockdown p53 using lentiviral vector pLKO.1. The expression levels of p53 were detected with western blot. (e) The expression levels of pri-miR-30a and mature miR-30a were analyzed by q-RT-PCR. The data were obtained from three times independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs Ctr.