Cellular, Molecular and Functional Characterization of the Tumor Suppressor Candidate MYO1C

Akademisk avhandling

Som för avläggande av medicine Doktorsexamen vid Sahlgrenska akademin vid
Göteborgs Universitet kommer att offentligen försvaras
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av

Kittichate Visuttijai

Fakultetopponent:
Professor Dr.Volker Haucke

Department of Molecular Pharmacology and Cell Biology
Leibniz-Institut für Molekulare Pharmakologie im Forschungsverbund
Berlin e.V. (FMP), Berlin, Germany

Avhandlingen baseras på följande arbeten:

I. Hedberg Oldfors C, Dios DG, Linder A, **Visuttijai K**, Samuelson E, Karlsson S, Nilsson S, Behboudi A: Analysis of an independent tumor suppressor locus telomeric to *Tp53* suggested *Inpp5k* and *Myo1c* as novel tumor suppressor gene candidates in this region. *BMC genetics* 2015, 16:80

II. **Visuttijai K**, Pettersson J, Mehrbani Azar Y, van den Bout I, Örndal C, Marcickiewicz J, Nilsson S, Hörnquist M, Olsson B, Ejeskär K, Behboudi A: Lowered expression of tumor suppressor candidate *MYO1C* stimulates cell proliferation, activates AKT and suppresses cell adhesion. *(2016) Submitted*

III. **Visuttijai K**, Faura Tellez G, Wettergren Y, Pettersson J, Hedberg Oldfors C, Behboudi A, and Ejeskär K. Expression of *MYO1C* is down-regulated in primary colorectal tumors. *(2016) Submitted*

IV. Pfister A, **Visuttijai K**, Sjöback R, Purvén M, Behboudi A, and Olsson B: Knockdown of MYO1C resulted in early activation of the PI3K/AKT pathway and disruption of key genes and pathways involved in apoptosis and cell cycle progression. *(2016) Submitted*

UNIVERSITY OF GOTHENBURG

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Kittichate Visuttijai

Department of Medical and Clinical Genetics, Institute of Biomedicine
Sahlgrenska Academy at University of Gothenburg, Sweden

Tumor suppressor genes play a role as a growth regulator and a gatekeeper of a cell. Their inactivation is often detected in malignant tumors. Identification of novel tumor suppressor gene candidates may help to further understand tumorigenesis and aid in the discovery of a new treatment leading toward cure of cancer.

This PhD research project aimed to understand functional significance of a novel tumor suppressor gene candidate, myosin IC (*MYO1C*) and to identify potential interaction(s) of the MYO1C protein with key components of the signaling pathways involving in cancer development.

In an experimental rat model for endometrial carcinoma (EC), detailed molecular genetic analysis of a candidate tumor suppressor region located distal to the tumor protein 53 (*Tp53*) suggested the myosin IC gene (*Myo1c*) as the best potential target for deletion of the genetic material. The question arising was whether and how *MYO1C* could function as a tumor suppressor gene. By using qPCR, Western blot or immunohistochemistry analyses, we examined MYO1C protein level in panels of well-stratified human colorectal cancer (CRC) and EC respectively. We found that MYO1C was significantly down-regulated in these cancer materials and that for the EC panel, the observed down-regulation of MYO1C correlated with tumor stage, where tumors at more advanced stages had less expression of MYO1C. In cell transfection experiments, we found that over-expression of MYO1C significantly decreased cell proliferation, and silencing *MYO1C* with siRNA increased cell viability. Additionally, knockdown of *MYO1C* impaired the ability of cells to migrate, spread and adhere to the surface. Recent published studies suggested a potential interplay between MYO1C and the phosphoinositide 3-kinase (PI3K)/AKT pathway. To examine this hypothesis, we analyzed the expression and/or activation of components of the PI3K/AKT and RAS/ERK signaling pathways *in vivo* in CRC samples, and *in vitro* in cells transfected with the *MYO1C* gene expression construct or *MYO1C*-targeted siRNA. To identify other potential pathways/ mechanisms through which MYO1C may exert its tumor suppressor activity, we additionally performed new sets of *MYO1C*-siRNA knockdown experiments. At different time points post transfection, we performed microarray global gene expression experiments followed by bioinformatics analysis of the data. Altogether, the results suggested an early PI3K/AKT response to altered *MYO1C* expression. We additionally identified several cancer-related genes/pathways with late response to *MYO1C* knockdown. All things considered, the identification of MYO1C-expression impact on cell proliferation, migration, and adhesion in combination with its interplay between several cancer-related genes and signaling pathways provide further evidence for the initial hypothesis of a tumor suppressor activity of MYO1C.

Keywords: MYO1C, myosin IC, tumor suppressor gene, cancer, tumor, PI3K/AKT signaling

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