REVERSAL OF THYROID DEDIFFERENTIATION AND AN INVASIVE PHENOTYPE BY SMALL MOLECULE KINASE INHIBITORS:

AN EXPERIMENTAL STUDY ON NORMAL AND MALIGNANT CELLS

Akademisk avhandling

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av

Camilla Ingeson Carlsson

Fakultetsopponent:

Professor James A Fagin, MD
Memorial Sloan-Kettering Cancer Center, Weill Cornell College, New York USA

Avhandlingen baseras på följande delarbeten:

I. **Ingeson Carlsson C, Nilsson M**
Switching from MAPK-dependent to MAPK-independent repression of the sodium-iodide symporter in 2D and 3D cultured normal thyroid cells
Mol Cell Endocrinol. 2013 Dec 5;381(1-2):241-54

II. **Ingeson Carlsson C, Nilsson M**
Dual contribution of MAPK and PI3K in epidermal growth factor-induced destabilization of thyroid follicular integrity and invasion of cells into extracellular matrix
Manuscript (submitted)

III. **Ingeson Carlsson C, Nilsson M**
Differential effects of MAPK pathway inhibitors on migration and invasiveness of $BRAF^{V600E}$ mutant thyroid cancer cells in 2D and 3D culture
Manuscript

UNIVERSITY OF GOTHENBURG

REVERSAL OF THYROID DEDIFFERENTIATION AND AN INVASIVE PHENOTYPE BY SMALL MOLECULE KINASE INHIBITORS:

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Camilla Ingeson Carlsson
Sahlgrenska Cancer Center, Institute of Biomedicine,
Sahlgrenska Academy at University of Gothenburg, Sweden

Abstract

Refractoriness to I-131 in dedifferentiated thyroid cancer is a great concern that restricts radioiodine therapy. There is also a lack of knowledge in understanding the mechanisms leading to repressed sodium iodide symporter (NIS) expression and impaired iodide uptake in tumor cells. With this background, *paper I* investigated how NIS and iodide transport in normal thyrocytes were affected during dedifferentiation induced by epidermal growth factor (EGF). This was done on highly differentiated thyroid epithelial cells cultured in low (0.5%) or high (5%) content of fetal bovine serum either on filter in bicameral inserts or embedded in 3D collagen gel. EGF abolished TSH-stimulated transcription of *NIS* in both type of cultures. U0126, a MEK inhibitor, reversed this effect but only in serum-starved 2D cultures. Inhibition of MAPK signaling failed to recover NIS-mediated iodide uptake in the presence of serum and in 3D-cultured follicles irrespective of serum. In contrast, EGF-induced down-regulation of thyroglobulin, the thyroid prohormone, was blocked by MEK inhibition. These findings suggest an additional mechanism besides the classical MAPK signaling that negatively regulates NIS and confer resistance to small molecule kinase inhibitors targeting the MAPK pathway in dedifferentiated thyroid cells.

In tumor progression cancer cells lose the ancestral epithelial phenotype and become invasive. Many mechanisms cooperate in this process including joint signaling of the MAPK and PI3K/AKT pathways, suggesting combined targeted treatment with kinase inhibitors would more effectively counteract invasiveness. This possibility was addressed in *paper II* in which cell migration into extracellular matrix from EGF-stimulated follicles was monitored during treatment with inhibitors of MEK (U0126) and PI3K (LY294002). Indeed, dual inhibition was required to prevent both cell proliferation and migration in response to EGF. Notably, single inhibition of PI3K adversely increased EGF-induced migration and invasion, probably by promoting disintegration of the follicular epithelium. As LY294002 did not compromise cell survival in the presence of EGF these findings call for caution in use of PI3K inhibitors as monotherapy of tumors with a constitutively activated MAPK pathway.

Activating $BRAF^{V600E}$ mutation is a common driver in thyroid cancer. Acquired drug resistance involving rebound activation of MAPK signaling restricts the promising possibility to treat BRAF mutant tumors with kinase-selective inhibitors as PLX4720. Combined drug treatment to overcome this is suggested. In *paper III* inhibitor efficacy on tumor cell migration was investigated in $BRAF^{V600E}$ mutant cell lines derived from papillary (BCPAP) and anaplastic (SW1736) thyroid cancer. Besides conventional scratch wounding a double-layered collagen gel model was developed for analysis of directed tumor cell invasion during prolonged culture. Both PLX4720 and U0126 inhibited BCPAP cell migration and reduced tumor cell viability in 3D culture. 2D migration of SW1736 cells resisted even combined drug treatment, whereas embedded in collagen gel both drugs reduced the invading cell numbers. However, dual inhibition of $BRAF^{V600E}$ and MEK did not prevent invasion although rebound activation of MAPK was blocked. This suggests presence of highly invasive tumor cell subclones in anaplastic cancer that escape targeted drug therapy due to MAPK independence.

Keywords: *Thyroid, cancer, NIS, MAPK, PI3K, $BRAF^{V600E}$, migration, 3D culture*

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