TARGETING mTOR COMPLEXES AND RIBOSOMAL S6 KINASES FOR THE DEVELOPMENT OF NOVEL THERAPIES FOR THYROID CANCER

Ioanna Koloni1,2, Christides Tsemaakis1,2,3, Christi Tsimpliou1, Evangelia Sereti1, Nikos Sakellaridis1, Aspasia Tzeou2, Konstantinos Dimas1

1Department of Pharmacology, Faculty of Medicine, Health Sciences School, University of Thessaly, Larissa, Greece
2Laboratory of Cytogenetics and Molecular Genetics, Faculty of Medicine, Health Sciences School, University of Thessaly, Larissa, Greece
3Equal contribution, Corresponding author: lkoloni@med.uvt.gr

Introduction

Thyroid Cancer is a malignant tumor in the thyroid gland, that is derived from follicular (Papillary Thyroid Cancer, Follicular Thyroid Cancer, Poorly Differentiated Thyroid Cancer and Anaplastic Cancer) or parafollicular (Medullary Thyroid Cancer) thyroid cells [1]. Studies have shown that the mTOR kinase and the ribosomal S6 kinase (S6K) family are key regulators of the PI3K/AKT and MAPK/ERK pathway respectively and adjust cell function and proliferation [2,3]. Dysregulation of the above pathways results in excessive cell proliferation, leading to carcinogenesis. The objective of this study was to investigate the role of mTOR and ribosomal S6 kinase (S6K) family as targets for new more effective therapeutic approaches for thyroid cancer.

mTOR Inhibitors

First generation inhibitors

Everolimus

Temsirolimus

Deformilimus

Second generation inhibitors

XL388

AZD2014

AZD8055

S6K Inhibitors

p90rsk inhibitor

BI-D1207

SL 0101-1

FMK

Tillotose

p70s6k inhibitor

GSK 2334470

BX-912

PF 4708671

Materials and Methods

In the present study, we investigated the development of patient-derived aggressive Papillary Thyroid Cancer cell population (APTC) [3] and Anaplastic Thyroid Cancer cell line (B505C) under the effect of specific inhibitors. In vitro SRA cytotoxicity assay was performed to investigate the effectiveness of mTOR, p70, p90 and PDK1 inhibitors in the above cell populations. CalcuSyn software was employed to study synergetic effect of combinations of the drugs [6]. Furthermore, the expression levels of the corresponding proteins were as well analyzed by Western Blot in cell extracts in an effort to compare them in both cell populations.

Results

Table 1. Growth Inhibitory 50 (G50), Total Growth Inhibitory (T50), Total Cyclin D (TCD) and Total Cyclin E (TCE) of mTOR inhibitors for B505C (Table 2a) and APTC (Table 2b). Second generation mTOR inhibitors exhibit better anti-proliferative activity (lower G50) and higher selectivity (higher T50) than first generation mTOR inhibitors. A438385 has the lowest G50 and highest T50 of all inhibitors tested. Furthermore, APTC seems to be more resistant to mTOR inhibitors than B505C.

Table 2a. Growth Inhibitory 50 (G50), Total Growth Inhibitory (T50), Total Cyclin D (TCD) and Total Cyclin E (TCE) of mTOR inhibitors for B505C (Table 2a) and APTC (Table 2b). First generation mTOR inhibitors exhibit lower anti-proliferative activity (higher G50) and lower selectivity (lower T50) than second generation mTOR inhibitors. A438385 has the highest G50 and lowest T50 of all inhibitors tested. Furthermore, APTC seems to be more resistant to mTOR inhibitors than B505C.

Conclusions

Under the experimental conditions of this study, second generation mTOR inhibitors exhibited better anti-proliferative activity and safety compared to first generation inhibitors. AZD8055 showed the greatest efficacy and safety of all inhibitors. Furthermore, APTC seems to be more resistant to mTOR inhibitors than B505C. Western Blot Analysis showed that most of mTORC1 and mTORC2 proteins are expressed to a greater extent in APTC compared to B505C. This could explain the greater resistance of APTC to mTOR inhibitors. B505C and APTC may exhibit significant efficacy in both cell types. PFT 470671 exhibited moderate activity. T-ac induced the highest efficacy amongst all S6K related inhibitors, both cell types were treated with this inhibitor to investigate its effect on S6K proteins. T-ac led to a small decrease in total p70 protein levels and reduced the expression of phospho-p70 (Ser223) residue, preventing its activation in both cell types. T-ac significantly reduced total p70 protein levels and phospho-p70 (Thr389) levels were increased in APTC. Finally, the combination of T-ac or GSX 2334470 exhibited synergistic anti-proliferative effect, suggesting that 6K and mTOR inhibitors may act synergistically towards a better therapeutic outcome for thyroid cancer.

References


Acknowledgements

This study was supported by the National Science Foundation under Grant Number 1247604 and the American Cancer Society under Grant Number 1247604.