Targeting oncogenic lncRNA and tumor cell metabolism to overcome drug resistance in BRAF-mutated thyroid carcinomas

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Background and rationale
Somatic mutations in BRAF gene activate distinct cellular pathways – if compared to mutations in RAS genes or other oncogenic alterations - in papillary thyroid carcinomas (PTCs) and associate with more severe tumor phenotypes and poor prognosis [1,2]. Tumor harboring BRAF_{V600E} mutation and rearrangements of RET oncogene (i.e. RET/PTC) show peculiar signature of oncogenic and tumor-suppressive long non-coding RNAs (lncRNAs), whose expression pattern is completely different compared to tumors driven by mutations in RAS genes or PPARG/PAX8 fusion [3]. Moreover, comparative transcriptome and methylome analysis of BRAF- and RAS-driven tumors reveal a massive reprogramming in the network of metabolic or metabolism-related genes occurring especially in BRAF-mutated samples. These data support the wide heterogeneity of PTCs, which results in differential approaches to the therapy. Indeed, the most common therapeutic option for treating BRAF-mutated PTC – especially at advanced or metastatic stages when the radioactive iodine uptake is abolished and surgery is not feasible [4] – as well as of BRAF-mutated anaplastic tumors (ATC), is represented by B-raf inhibitors (e.g. vemurafenib, VMR and dabrafenib, DBR). However, patients rapidly develop drug resistance because tumor cells activate alternative routes to induce MAPK pathway, bypassing B-raf inhibition [4-6]. Therefore, identifying new therapeutic strategies to overcome drug resistance in BRAF-driven thyroid tumors represents a primary need. Similar strategies may be adopted also in other BRAF-driven tumors, such as melanoma, colorectal and lung carcinomas.

Results
We are currently testing two different approaches to target BRAF-driven papillary and anaplastic thyroid carcinomas. One is based on the knockdown of the oncogenic thyroid-specific lncRNA COMETT, the other on the targeting of tumor cells’ metabolism to sensitize cells to standard chemotherapy.

1) We have recently identified the oncogenic lncRNA COMETT (Cytosolic Oncogenic Antisense To MET Transcript) as a transcript over-expressed in BRAF- and RET-driven PTCs and induced downstream the activation of MAPK signaling in cancer cells [3]. COMETT is part of a co-expression network that includes multiple oncogenes related to MAPK signaling, such as MAPK1, DUSP5 and MET). Interestingly, COMETT knockdown in vitro is sufficient to reduce the expression levels of many of them, resulting in a marked decrease in tumor cells’ viability, proliferation, motility/invasiveness and, more interestingly, in a higher sensitivity of tumor cells to the treatment with VMR. Hence, our recently published data [3] indicate COMETT lncRNA as a possible target to sensitize tumor cells to vemurafenib treatment.

2) Unpublished data indicate the presence of tumor subtype-specific alterations in the expression pattern of metabolic genes, characterized by a BRAF-specific glycolytic signature paralleled by the perturbation of TCA cycle and oxidative phosphorylation (OXPHOS) genes. These findings suggest that metabolic adaptation may represent a relevant Achille’s heel for tumor cells. To this aim, we have recently tested if - likewise melanoma [7] - B-raf inhibition can shift tumor cells toward OXPHOS, making them susceptible to metabolism-targeting compounds such as diclofenac [8,9]. Our preliminary results indicate that diclofenac acts synergistically with vemurafenib and markedly reduces PTC cell viability by repressing the expression of crucial glycolytic genes.

Future perspectives
The above-described approaches have provided intriguing results, so we decided to focus our future efforts to investigate toward both directions. On the one side, we are currently exploring the role of COMETT IncRNA in tumor cells (by RNA-pulldown and Mass Spectrometry to identify its protein partners). We also plan to test in vivo (by xenograft based on COMETT+ cells’ injection in immune-compromised NOD/SCID mice) whether COMETT silencing (by in vivo-stable ASO GapmeRs) can be proposed as new therapeutic option in advanced and drug resistant BRAF-mutated PTCs and ATCs in combination with VMR or DBR.

Finally, we are still evaluating in vitro how sensitize BRAF-mutant cell lines to currently available chemotherapy by targeting tumor metabolic pathways.

References:
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