of its expression. After demethylation, treatment of cells with TNF-related apoptosis-inducing ligand (TRAIL) led to the induction of apoptosis via activation of caspases-8, caspase-3, and poly(ADP-ribose) polymerase. Interestingly, gene silencing of *TMS1* using TMS1-specific small interfering RNA prevented TRAIL-mediated apoptosis.

Conclusion: Our results demonstrated that the *TMSI* gene is methylated in thyroid cancer cells and repression of methylation by 5-aza-2′-deoxycytidine restored expression of the *TMS1* gene and sensitized cells to TRAIL-induced apoptosis. These findings suggest that the *TMS1* gene can be targeted by combination of demethylating agents with TRAIL to induce efficient apoptosis in thyroid cancer cells.

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Mitochondrial Localization and Regulation of $BRAF^{V600E}$ in Thyroid Cancer: A Clinically Used RAF Inhibitor Is Unable to Block the Mitochondrial Activities of $BRAF^{V600E}$

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Context: The oncogenic $BRAF^{V600E}$ mutation results in an active structural conformation characterized by greatly elevated ERK activity. However, additional cellular effects caused by subcellular action of $BRAF^{V600E}$ remain to be identified.

Objective: To explore these effects, differences in the subcellular localization of wild-type and mutant BRAF in thyroid cancer were investigated.

Results: A significant proportion of endogenous and exogenous $BRAF^{V600E}$, but not wild-type BRAF, was detected in the mitochondrial fraction, similar to other BRAF mutants including $BRAF^{V600D}$, $BRAF^{V600K}$, $BRAF^{V600R}$, and $BRAF^{G469A}$, which showed elevated kinase activity and mitochondrial localization. Induced expression of $BRAF^{V600E}$ suppressed the apoptotic responses against staurosporine and TNFα/cycloheximide. Interestingly, the mitochondrial localization and antiapoptotic activities of $BRAF^{V600E}$ were unaffected by sorafenib and U0126 suppression of MAPK kinase (MEK) and ERK activities. Similarly, although the RAF inhibitor sorafenib effectively inhibited MEK/ERK activation, it did not block the mitochondrial localization of $BRAF^{V600E}$. In addition, inducible expression of $BRAF^{V600E}$ increased the glucose uptake rate and decreased O_2 consumption, suggesting that $BRAF^{V600E}$ reduces mitochondrial oxidative phosphorylation, a signature feature of cancer cells. Again, these metabolic alterations resulted by $BRAF^{V600E}$ expression were not affected by the treatment of thyroid cells by sorafenib. Therefore, RAF and MEK inhibitors are unable to block the antiapoptotic activity of $BRAF^{V600E}$ or correct the high glucose uptake rate and glycolytic activity and suppressed mitochondrial oxidative phosphorylation induced by $BRAF^{V600E}$.

Conclusions: The mitochondrial localization observed in oncogenic BRAF mutants might be related to their altered responses to apoptotic stimuli and characteristic metabolic phenotypes found in thyroid cancer. The inability of MEK and RAF inhibitors, U0126 and sorafenib, respectively, to block the mitochondrial localization of $BRAF^{V600E}$ has additional therapeutic implications for $BRAF^{V600E}$-positive thyroid cancers.

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