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RUNX3 can abrogate *CTNNB1* S45F mutation impaired apoptotic responses in desmoid tumors

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Abstract:

Objective: To investigate molecular driving forces behind the differences between the *CTNNB1* S45F and T41A mutations in DTs.

Methods: We conducted a gene array assay including desmoid tissues with *CTNNB1* T41A or S45F mutation. The gene array was validated using qRT-PCR. As an artificial system, we also transfected mutated β -catenin genes into normal embryonic cells (293T cells) to recapitulate the biology observed in desmoid cells. The ability of inducing apoptosis between the T41A and S45F mutated/transfected cells were assessed via flow cytometry analysis and via induction of cleaved caspase 3/7 after staurosporine or doxorubicin treatment.

Results: Our gene array analysis showed that proapoptotic genes are downregulated and anti-apoptotic genes are upregulated in the cells with the S45F mutation when compared to the T41A mutation. Moreover, we showed that there is no significant induction of apoptosis in the S45F-mutated desmoid cell strains or in the transfected 293T cells after staurosporine or doxorubicin treatment when compared to the T41A-mutated/transfected cells. Furthermore, our results showed a higher expression of nuclear β -catenin in the S45F-mutated cells when compared to the ones harboring the T41A mutation. Further investigating into these differences, we found that one of the pro-apoptotic genes downregulated in S45F-mutated tumors, *RUNX3*, has been shown to attenuate WNT signaling activity, suggesting that this gene could have an important role in the cross-talk between apoptosis and β -catenin pathway. To analyze the role of *RUNX3* in the response to apoptosis of S45F mutated desmoids we overexpressed *RUNX3* in the transfected 293T cells and then treated those cells with doxorubicin. Our results showed that the 293T *CTNNB1* S45F transfected with *RUNX3* have a higher induction of apoptosis when compared to the parental cells, suggesting that *RUNX3* plays a role in the resistance to apoptosis observed in the S45F mutated cells.

Conclusion: Taken together our results suggest that apoptosis is downregulated in desmoid tumors harboring the *CTNNB1* S45F mutation, and that cells with the *CTNNB1* S45F mutation are less able to undergo apoptosis than T41A-mutated cells. The impairment of apoptosis appears to be specific to the *CTNNB1* S45F mutation and not to desmoid tumors per se. Moreover, our results also showed that *RUNX3* may play a role in the resistance to apoptosis of S45F mutated cells and may be the cross-talk between the β -catenin and apoptosis pathway in desmoid tumors.