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Molecular mechanisms of the muscle differentiation blockade in Rhabdomyosarcoma

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Rhabdomyosarcomas (RMS) are common pediatric soft tissue tumors, show skeletal muscle features, unsatisfying responses to current therapies and a poor outcome that reflects the limited knowledge of RMS' pathogenesis. Since WNT signaling impacts skeletal muscle development, our preliminary expression study addressed this pathway in RMS cell lines, revealing over- expression of WNT5A, i.e. a ligand that activates the non-canonical branch of the WNT pathway in the less aggressive RME. Therefore, the current study had the following **aims:** 1. Assess the impact of WNT5A on functional features of RMS. 2. Investigate the crosstalk between the canonical and the non-canonical pathway in RMS and its impact on RMS features, particularly on skeletal muscle differentiation *in vitro.* 3. Figure out which non-canonical pathway plays a role in RMS tumorigenesis? 4. Assess the impact of overexpressed or downregulated non-canonical WNT signaling in RMS xenografts. 5. Determine whether WNT5A-mediated signaling affect the susceptibility of RMS towards an established, fAChR CAR T cell based immune therapy?
The spectrum of **experimental methods** comprised molecular (genes and proteins expression) and functional studies (proliferation, migration, invasion and sphere formation assays) of various representative RMS cell lines following stable WNT5A knock down and over- expression in vitro and an RMS xenograft model to validate the impact of WNT5A on RMS proliferation in vivo. The **key results** were the following: WNT5A attenuated RMS tumor cells proliferation, migration, invasion and sphere formation ability. These effects were accompanied by decreased ß-catenin nuclear localization and the expression of the associated transcription factor LEF1. In the xenograft model, faster tumor growth was observed for the WNT5A KD embryonal RMS cell line compared to control, whereas the alveolar RMS cell line didn't show any difference in tumor growth.
Additionally, WNT5A inhibited myogenic markers DESMIN and fetal AChR subunit gamma expression in RMS tumor cells, which made the RMS cells resistant to fAChR CAR-T cells. WNT5A effect on RMS tumorigenesis was associated with planar cell polarity (PCP) pathway regulation as WNT5A induced phospho-c-JUN expression.
In conclusion we hypothesized that the non-canonical WNT signaling pathway derived by WNT5A plays a role in RMS tumorgenesis and that it's associated with a more favorable RMS phenotype. These findings indicate that WNT5A could be a promising diagnostic and therapeutic target.