The αVβ3 integrin/NgR2 complex is upregulated in NEPrCa and is a novel therapeutic target

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Background: Neuroendocrine prostate cancer (NEPrCa) is a highly aggressive and metastatic subtype of PrCa. We have previously shown that NgR2 is upregulated by αVβ3, to which it associates, and promotes NE differentiation (NED) and upregulates RhoA, a protein associated with decreased disease-free survival after radical prostatectomy [1]. We also have previously shown that LM609, an αVβ3 integrin-specific inhibitory antibody, prevents NE tumor growth *in vivo* [2].

Methods: To analyze the expression of NgR2 in RNA PrCa patient databases, we collaborated with Drs. Dogra and Chen (Mount Sinai) [3]. Furthermore, in collaboration with Drs. Witte, Graeber, and Chen (UCLA), we used transcriptomic data from an organoid system [4], which allows us to follow the temporal evolution of NED from PrCa.

In a second set of experiments, we tested the ability of NgR2-expressing cells (NgR2-DU145) to promote tumor growth *in vivo;* immunohistochemical analysis (IHC) was also performed to analyze the expression levels of NgR2 as well as RhoA on NgR2-DU145 or MOCK-DU145 tumors and patient samples.

We then investigated whether the activation of RhoA in NEPrCa is NgR2 dependent, using Rho-activity assays.

Moreover, small extracellular vesicles (sEVs) were isolated from NgR2-DU145 cells (NgR2+sEVs) or Mock-DU145 cells (Mock-sEVs). Tumor growth of NgR2+sEVs or Mock-sEVs injected mice was measured, and IHC was performed to analyze NgR2 and Synaptophysin expression.

Finally, to test the αVβ3 integrin-directed targeting of NEPrCa-PDX tumors in SCID mice, iRGD and chemotherapeutic drugs were co-administered.

Results:

We show that a RNA sequencing database, characterized previously [3], shows significant enrichment of *RTN4RL2* (NgR2) in PrCa patient samples (n=5) compared to normal prostate tissue (n=5) (P=0.0095). We also demonstrate that NgR2 (*RTN4RL2*) is expressed in PrCa organoids [4], follows the temporal evolution of PrCa NED, correlates with the NE marker and inversely correlates with androgen receptor (*AR*) expression (n=10 per group) (P=0.001).

Furthermore, our *in vivo* analysis shows that NgR2 promotes tumor growth; the expression of NgR2 ($p=0.0366$) and RhoA ($p=0.0002$) is significantly higher in NgR2+ tumors (n=6) and correlates in NEPrCa patient samples (n=9).

We also show that RhoA is activated in NEPrCa in a NgR2-dependent manner ($P<0.001$).

We demonstrate that sEVs containing NgR2, when injected *in vivo* intratumorally, promote tumor growth and induce NED (n=9).

Finally, we used iRGD, a peptide which binds to αVβ3 integrin (overexpressed in NEPrCa) and can improve tumor treatment by increasing tumor targeting of chemotherapeutic drugs. This αVβ3 integrin directed treatment of the *in vivo* PDX mouse model by coadministration of iRGD plus carboplatin, does not increase the efficacy of carboplatin alone.

Conclusions: Our findings show that a novel pathway mediated by the αVβ3/NgR2 complex, which upregulates and activates RhoA, significantly contributes to NEPrCa progression, thus suggesting that this complex is a novel therapeutic target.

Publications:

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