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RESEARCH HIGHLIGHT

Autonomic nerve development contributes to prostate cancer progression

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In a significant translational study, Magnon *et al.* investigated the role that the autonomic nervous system plays in the development and spread of prostate cancer in both mice and human models. The study shows different roles for both branches of the autonomic nervous system, with the sympathetic system promoting early stages of tumorigenesis, and the parasympathetic system promoting cancer dissemination. This information could lead to important new foundations for treatment, therapies and management of prostate cancer.

In the last few years, there have been multiple studies that have shown evidence of tumor cell migration along nerves, a process termed perineural invasion. This perineural invasion has been correlated with a poorer prognosis in multiple cancers, including prostate.[1] Along similar lines, epidemiological studies recently noted that β-blocker therapy has been linked to lower recurrence rates and mortality when dealing with these cancers.[2] McVary *et al.*[3] have shown that the prostate is richly innervated with both sympathetic and parasympathetic branches, which help to control growth and maintenance of the prostate gland. Magnon *et al.*[4] having knowledge of this evidence, aimed to investigate the hypothesis of prostate tumor-infiltrating autonomic neonerves and their role in cancer development and dissemination.

Their methods included xenogeneic and transgenic cancer mouse models evaluating with bioluminescence, histology samples and positron emission tomography. They also retrospectively examined frozen human prostate samples from 43 patients.

They started by investigating the sympathetic system and its relation to prostate cancer. After injecting PC-3 human prostate cells into mice, they evaluated the samples 11 weeks later with bioluminescence. Results showed both tumor-infiltrating sympathetic fibers (adrenergic) and intratumor parasympathetic fibers (cholinergic) throughout the prostate and tumor. Using tumor markers NF-L and NF-H, which mark newly formed and mature nerve fibers, stains showed an abundance of NF-L, suggesting the tumor recruits newly formed nerves into the stroma.

To investigate the function of the sympathetic fibers, they ablated them with 6OHDA, a chemical which specifically destroys TH^+ nerve fibers, sparing the parasympathetic $VAChT^+$ epithelial nerve fibers, while also shown not be toxic to tumor cells based on controls. Ablation prevented the development of tumors in the prostate, suggesting a sympathetic role in tumor engraftment. They also showed no detectable metastasis compared to the PC-3 mice 11 weeks after injection, again likely due to impaired tumor development. To account for local signals in the tumor microenvironment, the hypogastric nerves, which deliver sympathetic signals to mice prostates, were surgically removed in another cohort of mice. The surgical denervation showed markedly inhibited tumor development when compared to intact nerve mice by week 4. All of this suggests that the sympathetic signals are critical at the early stages in the mouse model.

Other studies have shown that β2 adrenergic receptors enhance tumor growth.[5] To investigate this, mice were bred deficient in β2, β3 or both receptors. The mice lacking a single receptor showed slightly delayed tumor development, but if both receptors were missing, tumor development was severely inhibited. This reinforces the importance that the adrenergic receptors also play in the tumor microenvironment.

To account for a more genetic model of prostate cancer, the researchers also induced cMyc overexpression in some of the mice, which led to a complete penetrance of prostate cancer. Using these mice, chemical sympathectomy was performed at various times in development. If the sympathectomy occurred at 2 days, the prostate intraepithelial neoplasia incidence was decreased by 83%. At 1 month, there was a 25% reduction. And at 2 months, no reduction was seen in the incidence.

Due to finding the $VAChT^+$ parasympathetic fibers in samples, the team also wanted to assess their role. Ach was previously reported to induce proliferation of prostate cancer, and the *Chrm1* receptor was found to be expressed more in healthy human prostate tissues.[6] The cells were treated with carbachol, a *Chrm* agonist. It was found that carbachol treatment significantly increased the tumor cell invasion into the pelvic lymph nodes. When treated with scopolamine or pirenzepine, which are *Chrm1* antagonists, lymph node invasion was inhibited. Overall, carbachol treatment showed significantly increased incidence of prostate intraepithelial neoplasia, and accelerated progression. There was overall a factor of 6 increase in tumor metastasis when compared to control. When the environment was deprived of *Chrm1*, even with carbachol treatment, the lymph node spread was significantly reduced, and signaling did not affect the growth of the prostate. This suggested that *Chrm1* affects the dissemination and proliferation of the tumor.

After discovering the roles of nerve fibers based on mice models, human models were also investigated. Retrospectively, 43 human prostatectomy tissues were evaluated. They were separated into a low risk group ($n=30$, prostate-specific antigen <10, Gleason score <7, stage T1c or T2a) or a high risk group ($n=13$, prostate-specific antigen >10, Gleason score >7, stage $>$T2b). It was found that, in the high-risk group patients, a higher nerve fiber density was seen when evaluated with

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staining. It was also seen that these patients had a higher tumor proliferative index. There was also a positive correlation between higher nerve density and both biochemical recurrence and tumor spread outside of the prostate.

In the end, a number of significant insights into prostate cancer were seen. The team had found that adrenergic fibers from sympathetic nerves, acting via β2 and β3 receptors, are important in the initial phases of tumor cell development, as well as promoting tumor cell survival. This is consistent with the role that beta blockers have in helping improve survival of cancer patients, as seen in recent studies. The parasympathetic nervous system was found to play a role in tumor cell invasion, migration and distant metastasis using *Chrm1*-mediated signals. Supporting the mouse model, the human prostate specimens supported this, showing higher nerve fiber densities in the higher risk patients with higher grades of prostate cancer. With the understanding of prostate cancer behavior dicoctomized in such a clear fashion, this work may shed new insight for new ways to prevent, treat and manage patients with prostate cancer.

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