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RESEARCH HIGHLIGHT

Does the autonomic nervous system contribute to the initiation and progression of prostate cancer?

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In the July 12 issue of *Science* magazine, researchers from the Albert Einstein College of Medicine, the Mount Sinai School of Medicine, the Durham VA Medical Centre and Duke University published an elegant study demonstrating that the sympathetic nervous system, acting through β_2 and β_3-adrenoceptors in the prostate, plays an important role in the initiation of prostate cancer, while the parasympathetic nervous system plays a role in the dissemination of tumour metastases via M_1 muscarinic receptors. These findings are significant because they indicate that receptors associated with the autonomic nervous system may be viable targets for prostate cancer therapy.

The influence of the autonomic nervous system on physiological systems changes with age. In particular, the sympathetic nervous system is implicated in disorders of the aging male, including hypertension and benign prostatic hyperplasia. In both disorders, the sympathetic nervous system shows signs of overactivity and symptoms can be controlled by the use of therapeutic drugs which block the effects mediated by adrenoceptors (e.g., α_1-adrenoceptor antagonists, β-blockers). It has been hypothesized previously that there may be a similar association between increased sympathetic activity and prostate cancer.[1,2]

Cells innervated by the sympathetic nervous system respond to the neurotransmitter noradrenaline via nine different adrenoceptor (AR) subtypes, the α_{1A}-AR, α_{1B}-AR and α_{1D}-AR, three α_2-AR subtypes, and β_1-AR, β_2-AR and β_3-ARs. These receptors are also activated by adrenaline released from the adrenal gland in response to behavioural stress. There is robust evidence linking activation of the β_2-AR with progression and/or metastasis of breast cancer,[3] ovarian cancer[4,5] and acute lymphoblastic leukaemia,[6] but these studies have focused on animals with high circulating levels of adrenaline. A recent study has also shown that in mouse models of prostate cancer, immobilisation stress and systemic adrenaline promote prostatic intraepithelial neoplasia (PIN) and resistance of tumours to apoptosis normally induced by the PI3K inhibitor ZSTK474.[7]

Unlike studies based on tumour xenografts in the flank of immunocompromised mice, the work described by Magnon *et al.*[8] employed orthotopic injection of human PC-3 prostate cancer cells directly into the ventral prostate gland, mirroring the tumour microenvironment found in human disease. The PC-3 cells were labelled with luciferase so that they could be visualized and quantified *in vivo* or *ex vivo* using bioluminescence imaging. Tumours were readily detected after 5 weeks. At 11 weeks, tumour-infiltrating sympathetic fibres arising from the surrounding normal tissue were apparent (characterized by positive staining for the noradrenaline-synthesizing enzyme tyrosine hydroxylase), as were intratumour parasympathetic fibres identified by positive staining for vesicular acetylcholine transporter.

Both chemically-induced sympathectomy using 6-hydroxydopamine, or surgical sympathetic denervation, prevented the development of tumours in the prostate. The target cells for sympathetic stimulation were shown to be stromal cells rather than the PC-3 tumour cells, as recipient mice with genetic deletion of β_2-/β_3-ARs also displayed a marked decrease in tumour development. This is interesting, as the previous study on prostate cancer[7] indicated that β_2-ARs expressed by tumour cells mediate the effects of behavioural stress. There are key differences between these studies,[7,8] namely, the sites of tumour injection (orthotopic injection *vs.* flank xenograft), the stimulus investigated (increased sympathetic innervation of the prostate *vs.* acute behavioural stress leading to increased circulating adrenaline), and the time course of the measured responses (resistance to apoptosis over a period of 6–72 h *vs.* tumour development over the course of 11 weeks).

Magnon *et al.*[8] also investigated the functional significance of parasympathetic nerve infiltration in prostate tumours. Stimulation of cholinergic pathways using the acetylcholine receptor agonist carbachol promoted metastasis rather than affecting primary tumour development. This effect on metastasis was blocked by the nonselective muscarinic receptor antagonist scopolamine as well as the M_1 muscarinic receptor antagonist pirenzepine. In immunocompromised mice with genetic deletion of the M_1 receptor, carbachol no longer promoted metastasis. Again, cells within the recipient prostate rather than the injected tumour cells mediated the effects of parasympathetic stimulation.

The findings above were confirmed in Hi-Myc mice that spontaneously develop prostate cancer.[9] Hi-Myc mice displayed PIN by 2 weeks after birth, progressing to invasive adenocarcinomas within 6 months. Chemical sympathectomy of Hi-Myc mice prior to one month of age led to greatly reduced tumour development, whereas surgical or chemical sympathectomy at 2 or 5 months had no effect on PIN or invasive cancer, indicating that the sympathetic nervous system is important primarily in the initiation and early stages of prostate tumour development. In contrast, Hi-Myc mice treated with carbachol even at 3 months of age displayed substantial increases in PIN and progression to invasive lesions. These effects of carbachol were abolished in M_1 knockout mice or in wild-type mice treated with the antagonist pirenzepine. Thus in both Hi-Myc animals and immunocompromised mice injected with PC-3 cells, sympathetic and parasympathetic activity

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within the prostate promote disease progression via complementary but distinct mechanisms. This characteristic of malignant disease is not observed in normal physiology, as the sympathetic and parasympathetic nervous systems generally produce opposing responses. In normal prostate gland, for example, parasympathectomy leads to increased prostate weight, while sympathectomy inhibits growth.[10]

Magnon *et al.*[8] addressed the clinical relevance of their mouse data by examining prostatectomy samples taken from 43 treatment-naive patients with prostate cancer. Patients were classified as having low (30) or high (13) risk based on prostate-specific antigen levels, Gleason score and disease stage. Prostate tissue surrounding the cancers in high-risk patients was found to have increased noradrenergic fibre density, while cholinergic fibres were restricted to the tumours. A high density of both noradrenergic and cholinergic fibres was associated with a higher proliferative index. Interestingly, correlations were also found between nerve fibre density and preoperative prostate-specific antigen levels, time until recurrence and tumour spread. The authors suggest that assessment of autonomic nerve density may provide a useful diagnostic tool for prediction of tumour aggressiveness. This would be a major prognostic advance, as it may be feasible to use more conservative treatment regimes in patients with low nerve density combined with low risk based on Gleason score and prostate-specific antigen levels. Such assessment would also highlight high-risk patients most likely to benefit from treatment with β-blockers or muscarinic antagonists.

Recent epidemiological evidence is consistent with the findings of this study, as treatment with β-blockers is associated with improved survival of prostate cancer patients.[11,12] However, both clinical studies show a β-blocker effect on high-grade, aggressive and metastatic tumours, in contrast to the tumour initiating effect of β-adrenoceptors in the mouse study.[8] Furthermore, it is noteworthy that most clinically used β-blockers are somewhat selective for the β_1-AR subtype, in contrast to the β_2- and β_3-AR subtypes found to initiate tumours in the mouse study. Note that all three adrenoceptor and muscarinic receptor subtypes investigated in this study were involved in tumour initiation and/or progression. There is also epidemiological evidence to suggest that α_1-AR antagonists may be of benefit in prostate cancer,[13,14] so their involvement in disease development also requires investigation.

Given the findings of this study,[8] the targeting of G protein-coupled receptors associated with the autonomic nervous system for the treatment of prostate and other cancers may be a viable proposition. This is exciting, as potent and selective antagonists for these receptors are already available and generally well tolerated in patients, in contrast to the severe side effects associated with commonly used chemotherapeutic agents. The authors of the present study have not ruled out contributions by other G protein-coupled receptor subtypes; thus, future studies should investigate additional receptors as well as the signalling pathways that they activate, as these may offer even more novel therapeutic targets for the treatment of cancer.

1 Rao J, Yang J, Liu Z, Wang L, Yin Z *et al*. Hypothetic association between greater sympathetic activity and prostate cancer. *Med Hypotheses* 2008; **71**: 442–3.
2 White CW, Xie JH, Ventura S. Age-related changes in the innervation of the prostate gland: implications for prostate cancer initiation and progression. *Organogenesis* 2013; **9**: 206–15.
3 Sloan EK, Priceman SJ, Cox BF, Yu S, Pimentel MA *et al*. The sympathetic nervous system induces a metastatic switch in primary breast cancer. *Cancer Res* 2010; **70**: 7042–52.
4 Armaiz-Pena GN, Allen JK, Cruz A, Stone RL, Nick AM *et al*. Src activation by beta-adrenoreceptors is a key switch for tumour metastasis. *Nat Commun* 2013; **4**: 1403.
5 Thaker PH, Han LY, Kamat AA, Arevalo JM, Takahashi R *et al*. Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nat Med* 2006; **12**: 939–44.
6 Lamkin DM, Sloan EK, Patel AJ, Chiang BS, Pimentel MA *et al*. Chronic stress enhances progression of acute lymphoblastic leukemia via beta-adrenergic signaling. *Brain Behav Immun* 2012; **26**: 635–41.
7 Hassan S, Karpova Y, Baiz D, Yancey D, Pullikuth A *et al*. Behavioral stress accelerates prostate cancer development in mice. *J Clin Invest* 2013; **123**: 874–86.
8 Magnon C, Hall SJ, Lin J, Xue X, Gerber L *et al*. Autonomic nerve development contributes to prostate cancer progression. *Science* 2013; **341**: 1236361.
9 Ellwood-Yen K, Graeber TG, Wongvipat J, Iruela-Arispe ML, Zhang J *et al*. Myc-driven murine prostate cancer shares molecular features with human prostate tumors. *Cancer Cell* 2003; **4**: 223–38.
10 McVary KT, Razzaq A, Lee C, Venegas MF, Rademaker A *et al*. Growth of the rat prostate gland is facilitated by the autonomic nervous system. *Biol Reprod* 1994; **51**: 99–107.
11 Grytli HH, Fagerland MW, Fossa SD, Tasken KA. Association between use of β-blockers and prostate cancer-specific survival: a cohort study of 3561 prostate cancer patients with high-risk or metastatic disease. *Eur Urol* 2003; in press.
12 Grytli HH, Fagerland MW, Fossa SD, Tasken KA, Haheim LL. Use of β-blockers is associated with prostate cancer-specific survival in prostate cancer patients on androgen deprivation therapy. *Prostate* 2013; **73**: 250–60.
13 Harris AM, Warner BW, Wilson JM, Becker A, Rowland RG *et al*. Effect of alpha$_1$-adrenoceptor antagonist exposure on prostate cancer incidence: an observational cohort study. *J Urol* 2007; **178**: 2176–80.
14 Murtola TJ, Tammela TL, Maattanen L, Ala-Opas M, Stenman UH. Prostate cancer incidence among finasteride and alpha-blocker users in the Finnish Prostate Cancer Screening Trial. *Br J Cancer* 2009; **101**: 843–8.