Supporting Information

An on-chip model for investigating the interaction between neurons and cancer cells

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Experiments

Detection of neurotransmitters in cultured DRG neurons

The neurotransmitters released from DRG neurons, including norepinephrine (NE) and acetylcholine (ACh), were quantified by enzyme-linked immunosorbent assay (ELISA). NE was detected using norepinephrine ELISA kit (Abnova). ACh was detected with choline/acetylcholine kit using fluorometric assay (Abcam).

Detection of adrenergic receptors and muscarinic receptors on PC-3 cancer cells

The expression of beta-adrenergic receptors (β-ARs) and muscarinic receptors (mAChRs) in PC-3 cells were evaluated by Western blot assay. In brief, lysates of cells were prepared with lyses buffer, separated on 10% SDS-polyacrylamide gel, and transferred to a PVDF membrane. The membrane was blocked with 5% non-fat milk, 0.1% Tween 20 in PBS for 30 min at room temperature, and incubated with primary antibody at 4°C overnight. The primary antibodies included anti-beta 1 adrenergic receptor pAb (β_1-AR, Abcam), anti-beta 2 adrenergic receptor pAb (β_2-AR, Abcam), anti-muscarinic acetylcholine receptor 1 (m_1AchR, Abcam) and anti-muscarinic acetylcholine receptor 3 (m_3AchR, Abcam). GAPDH was used as an internal control. The membranes were then incubated by secondary antibody conjugated to horseradish peroxidase (HRP) (Jackson) at 37 °C for 1 h. The Western blot signals were detected using an enhanced chemiluminescence system (Amersham, GE Healthcare), and the intensity of proteins was quantified using a Bio Imaging System.

Results

Sensitivity and specificity of the neuron-cancer crosstalk

To prove the sensitivity and specificity of the neuron-cancer crosstalk in the on-chip model (Fig. 6A), we compared the effect of agonist and antagonist on the NE-beta-adrenergic signaling and muscarinic signaling, respectively. We applied beta agonist (isoproterenol, 10 μM) and beta blocker (propranolol, 10 μM) on the PC-3 cells to active or block the NE-beta-adrenergic signaling, respectively. As compared to the non-treated PC-3 cells, the stimulation of NE-beta-adrenergic signaling by isoproterenol effectively enhanced the migration of PC-3 cells along the neurites, whereas the blocking of NE-beta-adrenergic signaling by propranolol suppressed the migration of the PC-3 cells along the neurites (Fig. S7A). Similarly, the activation of muscarinic signaling by muscarinic agonist (carbachol, 10 μM) enhanced the PC-3 cell migration along the neurites, whereas the blocking of muscarinic signaling by muscarinic antagonist (hyoscine, 10 μM) impaired the migration of PC-3 cells (Fig. S7B). These results prove the sensitivity and specificity of the neuron-cancer crosstalk in the on-chip model.

Supplementary Figures

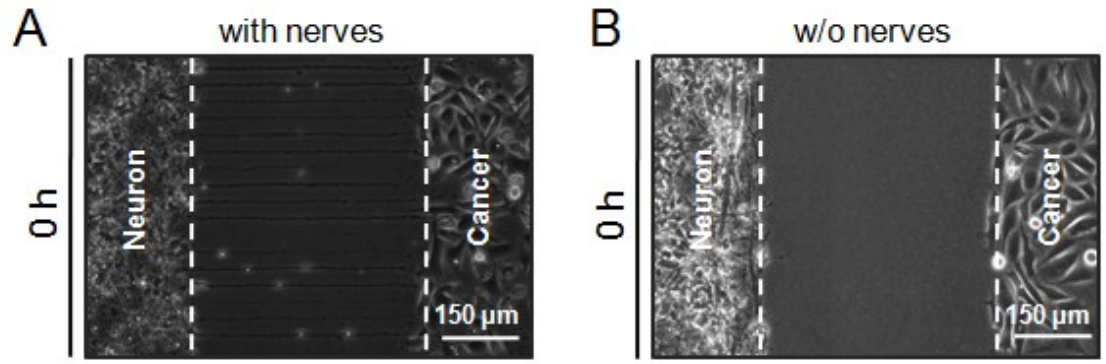

Fig. S1 Investigation of nerve-cancer crosstalk in the microfluidic chip. Representative phase-contrast images illustrate the nerve-cancer coculture with (A) an without (B) the presence of DRG neurites in the interconnected regions, at the time point of the removal of the PDMS cover (0 h).

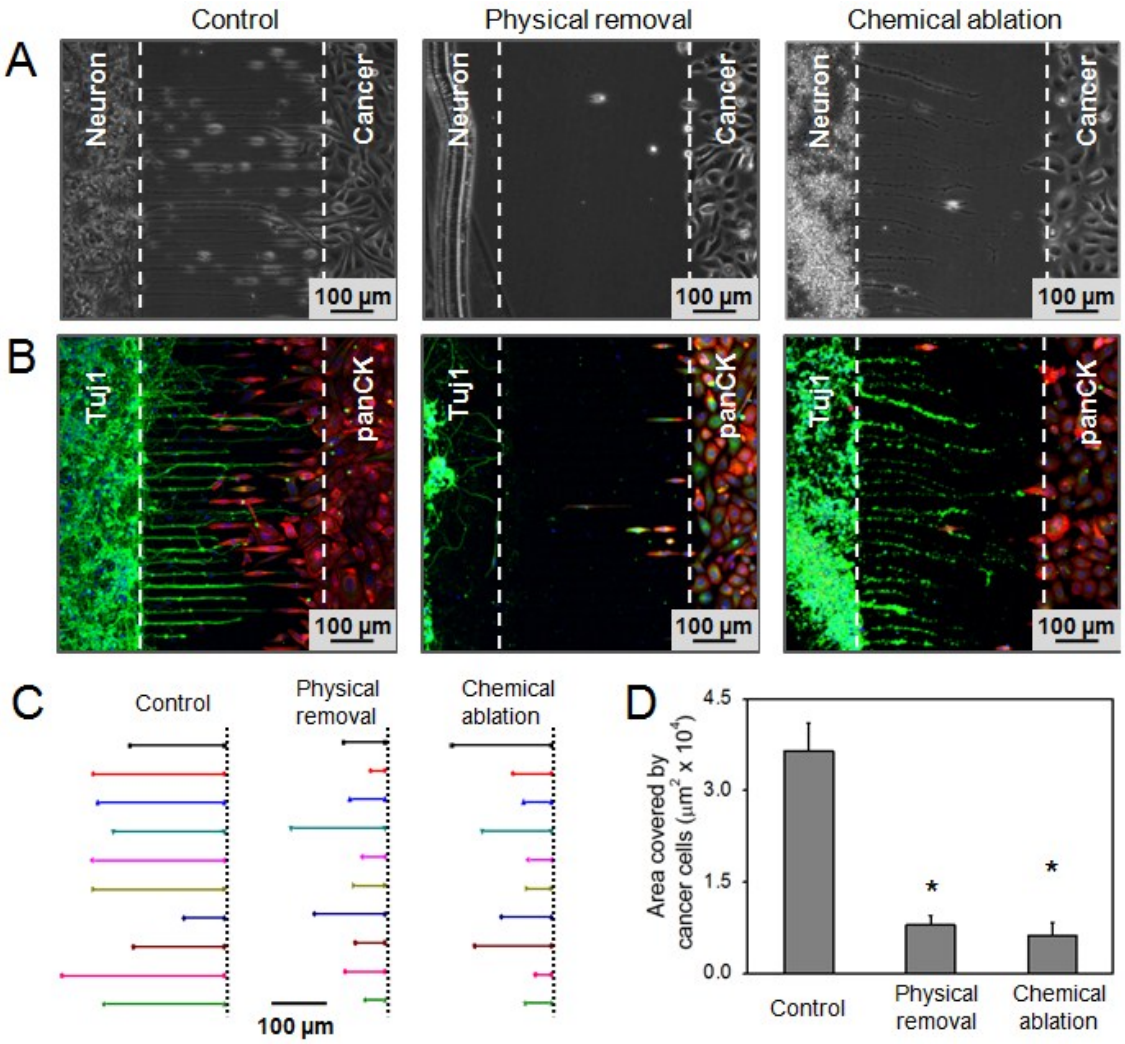

Fig. S2 Ablation of neurites reduces the migration of PC-3 cells along neurites. (A) Phase-contrast images and (B) immunofluorescence images of DRG neurons and PC-3 cells co-cultured in the microfluidic chip at 24 h after peeling off the PDMS cover, (left panel) control with the presence of the neurites, (middle panel) physical removal of the neurites, and (right panel) chemical ablation of neurons by 6-OHDA in the interconnected regions. Neuron, cancer cells, and nuclei are stained with Tuj1 (green), panCK (red) and DAPI (blue), respectively. (C) Traces of PC-3 cells at 24 h intervals with different treatments as indicated in (A-B). (D) Quantification of the surface area covered by migrated PC-3 cell projections in the interconnected regions, at 24 h after removal of the PDMS cover (each counted field surface is 0.30 mm^2). * $p < 0.01$.

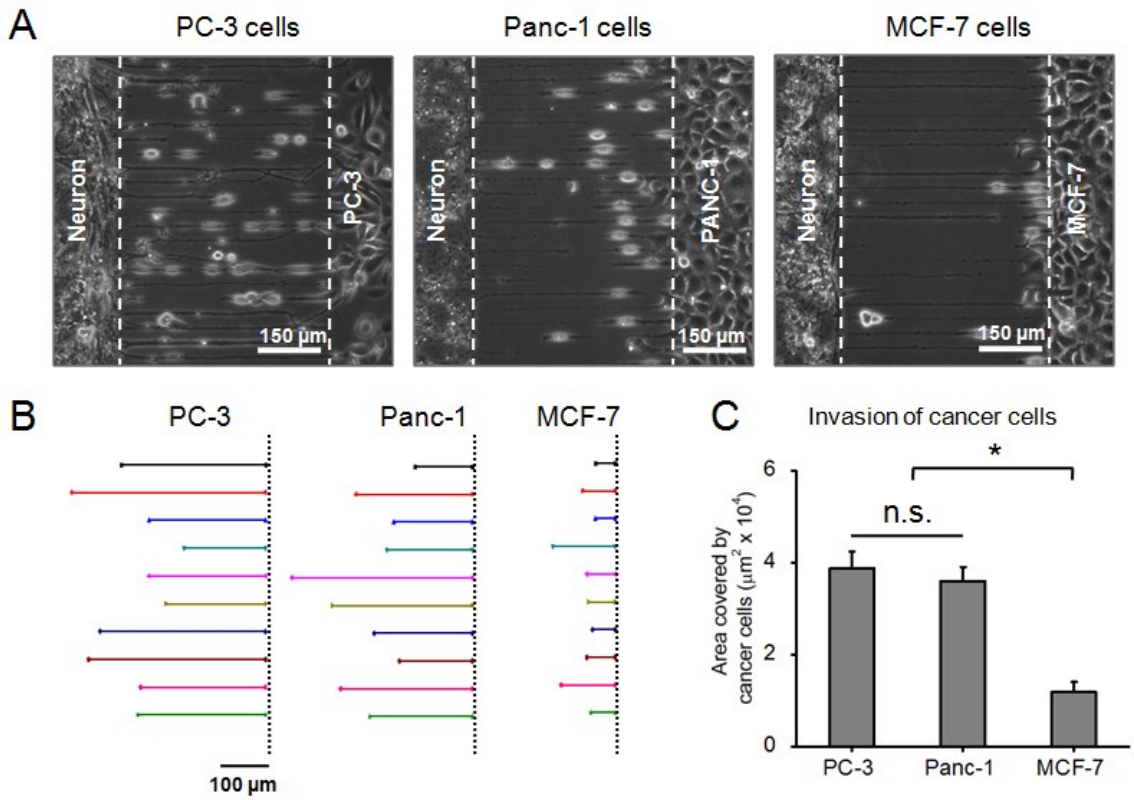

Fig. S3 Simulation of cancer cell migration along neurites with different types of cancer cells. (A) Phase-contrast images of DRG neurites interact with different types of cancer cells at 24 h after removal of the PDMS covers, with PC-3 prostate cancer cells, Panc-1 pancreatic cancer cells, and MCF-7 breast cancer cells. (B) Traces of different cancer cells at 24 h intervals after removal of the PDMS covers. (C) Quantification of the surface area covered by migrated cancer cell projections in the interconnected regions. Each counted field surface is 0.30 mm^2. * $p < 0.01$.

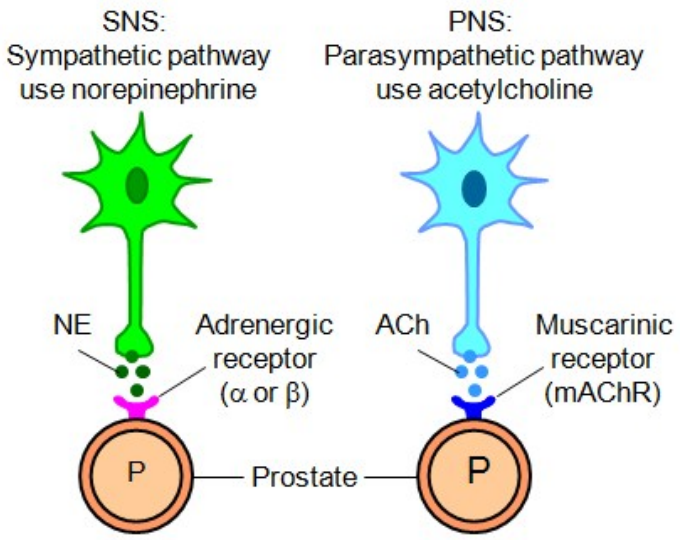

Fig. S4 Schematic presentation of innervation and neural signaling in prostate. Autonomic nervous system (ANS) consists of sympathetic nervous system (SNS) and parasympathetic nervous system (SNS). At the prostate interface, sympathetic nerves release norepinephrine (NE), which binds to adrenergic receptors (α or β) on stromal cells of prostate, whereas parasympathetic nerves release acetylcholine (ACh), which acts on muscarinic receptor (mAChR) on stromal cells of prostate. "P" indicates prostate.

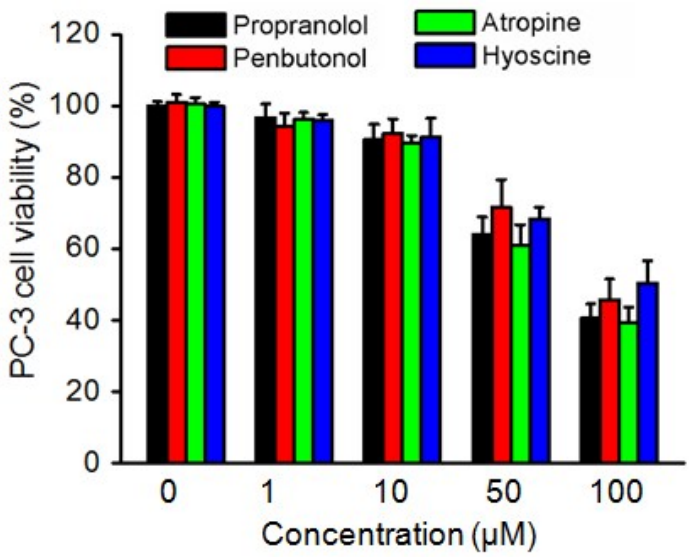

Fig. S5 PC-3 cell viability after incubation overninght with β-blockers (propranolol and penbutolol) and muscarinic antagonists (atropine and hyoscine) with different concentrations between 0 and 100 μM.

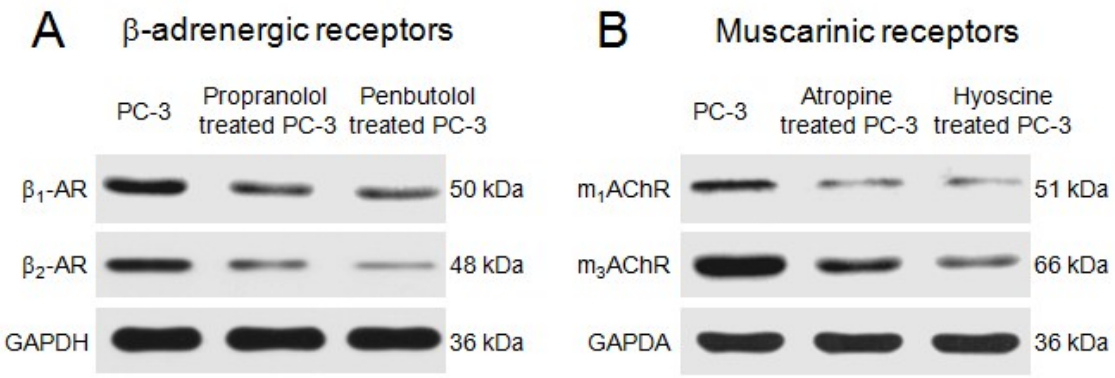

Fig. S6 Western blot analysis of β-adrenergic receptors and muscarinic receptors on PC-3 cells. (A) β-adrenergic receptors (β-ARs) were detected by Western blot analyses with specific polyclonal antibodies to β_1-AR and β_2-AR subtypes. Major bands for the β_1-ARs and β_2-ARs were detected at 50 and 48 kDa, respectively. (B) Muscarinic receptors (mAChRs) were detected by Western blot analyses with specific antibodies to m_1AChR and m_3AChR subtypes. Major bands for the m_1AChR and m_3AChR were detected at 51 kDa and 66 kDa, respectively. GAPDH was used as an internal control.

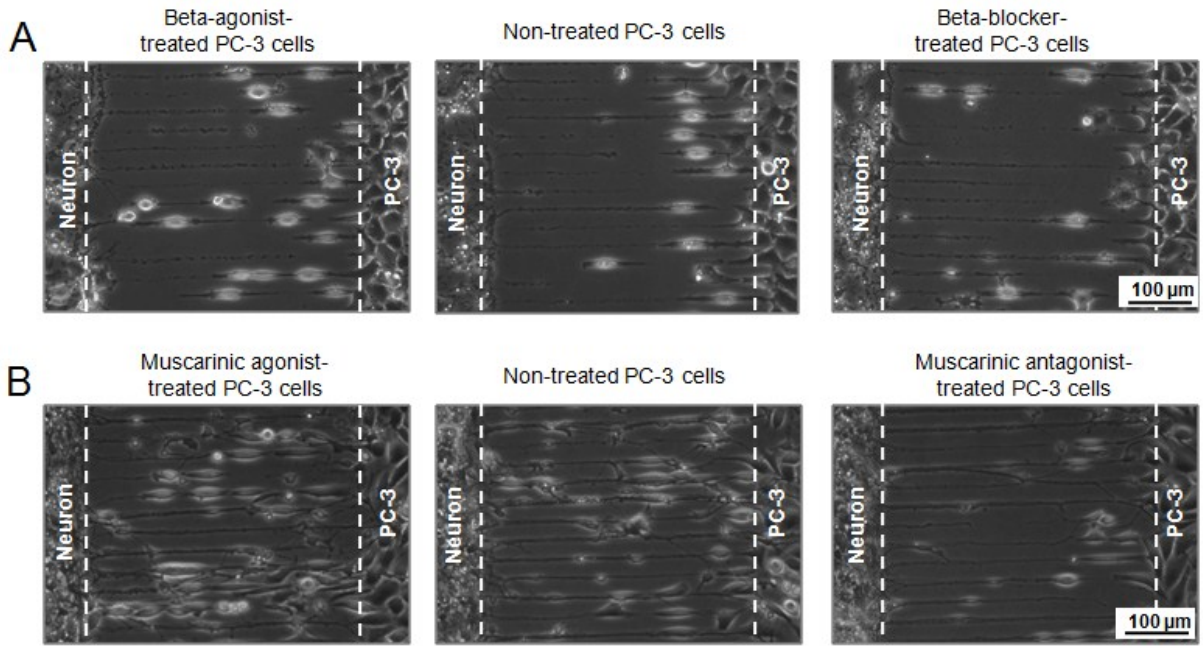

Fig. S7 Sensitivity and specificity of the neuron-cancer crosstalk in the on-chip model. (A) Migration of PC-3 cells at 18 h after peeling off PDMS covers, with pre-treatment of PC-3 cells with beta-agonist (isoproterenol, 10 μM) and β-blocker (propranolol, 10 μM), respectively. (B) PC-3 cell migration at 18 h after peeling off PDMS covers, with PC-3 cells pre-treated with muscarinic agonist (carbachol, 10 μM) and muscarinic antagonist (hyoscine, 10 μM), respectively. Non-treatreated PC-3 cells served as control groups.

Supplementary Tables

Table S1 Quantification of neurotransmitters released by DRG neurons.

	DRG neurons	6-OHDA-treated DRG
Norepinephrine	14.4 ± 2.9 nmol/L	1.6 ± 0.4 nmol/L
Acetylcholine	612 ± 69 nmol/L	57 ± 19 nmol/L

Supplementary Movies

Movie S1 Cancer cell disengaged from the cancer compartment and migrated along the contacted neurites.

Movie S2 Cancer cell navigated along the contacted neurites for migration forward.

Movie S3 Cancer cell navigated along the neurites and jumped to the adjacent neurites for migration forward.

Movie S4 Cancer cell migrated backward along the contacted neurites.

Movie S5 Cancer cell migration without the contact to neurites.

Movie S6 Cancer cell migration without the presence of neurites.