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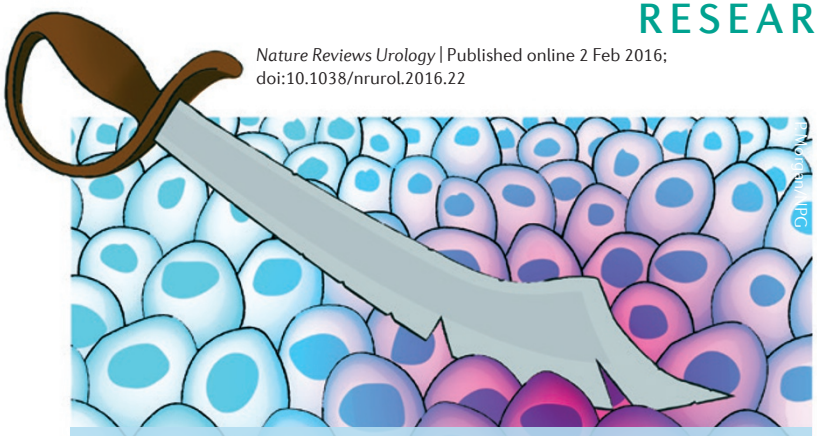

BLADDER CANCER

To be or NOTCH2 be?

NOTCH2 acts as an oncogene to promote growth and metastasis of bladder cancer, and could be specifically targeted for therapy, according to a study in *Clinical Cancer Research*.

NOTCH is a family of transmembrane receptors known to be involved in differentiation, proliferation and invasion. Alterations in NOTCH signalling have been shown in bladder cancer; however, previous studies have focussed on *NOTCH1* — a tumour suppressor. "Previous articles published on Notch in bladder cancer determined that *Notch* is a tumour suppressor, but they all focused on *Notch1*," explains corresponding author Peter Black. "By writing that *Notch* is a tumour suppressor, without specifying that this is really specific to *Notch1* (and likely *Notch3*), important detail is lost."

By analysing the published data from The Cancer Genome Atlas (TCGA) for aberrations in *NOTCH1*, *2* and *3* in bladder cancer, Black's team found that *NOTCH2* was often gained, whereas *NOTCH1* and *NOTCH3* were often deleted. Furthermore, patients whose tumours showed high NOTCH2 expression had a significantly worse prognosis than those with low or moderate expression. NOTCH2-overexpressing tumours were often basal subtype, with increased epithelial–mesenchymal transition (EMT) and higher stem cell marker expression. Immunohistochemical analysis on muscle-invasive bladder cancer samples showed NOTCH2 expression in 72% of bladder tumours.

In order to assess the function of NOTCH2, the team transduced two epithelial cell lines with a lentiviral construct coding for the *NOTCH2* intracellular domain (N2ICD). N2ICD-overexpressing cell lines had a higher invasive ability than mock-transduced cells. Furthermore, mesenchymal marker expression was increased, whereas epithelial marker expression was decreased on western blot compared with controls. N2ICD cell lines also showed increased proliferation and cell cycle progression.

Testing these effects in a xenograft model using bioluminescent imaging, they showed that mice inoculated with N2ICD+ cells exhibited higher bioluminescence than mock-cell-inoculated mice, suggesting increased tumour volume. Expression of mesenchymal versus epithelial markers once again suggested an increase in EMT characteristics.

The team went on to test whether NOTCH2 inhibition could have a therapeutic role using stable knockdown of *NOTCH2* in cell lines using short hairpin (sh)RNA. Knockdown resulted in a decrease in invasive ability and reduced growth and colony formation compared with control cells. In orthotopic xenografts using these cells, silencing of *NOTCH2* inhibited tumour growth compared with control shRNA xenografts.

"The different roles of Notch1 and Notch2 in bladder cancer mean that pan-Notch inhibitors are unsuitable for therapy. Instead a specific Notch2 inhibitor would be more rational," Black concludes.

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ORIGINAL ARTICLE(S) Hayashi, T. *et al.* Not all NOTCH is created equal: the oncogenic role of NOTCH2 in bladder cancer and its implications for targeted therapy. *Clin. Cancer Res.* http://dx.doi.org/10.1158/1078-0432.CCR-15-2360 (2016)