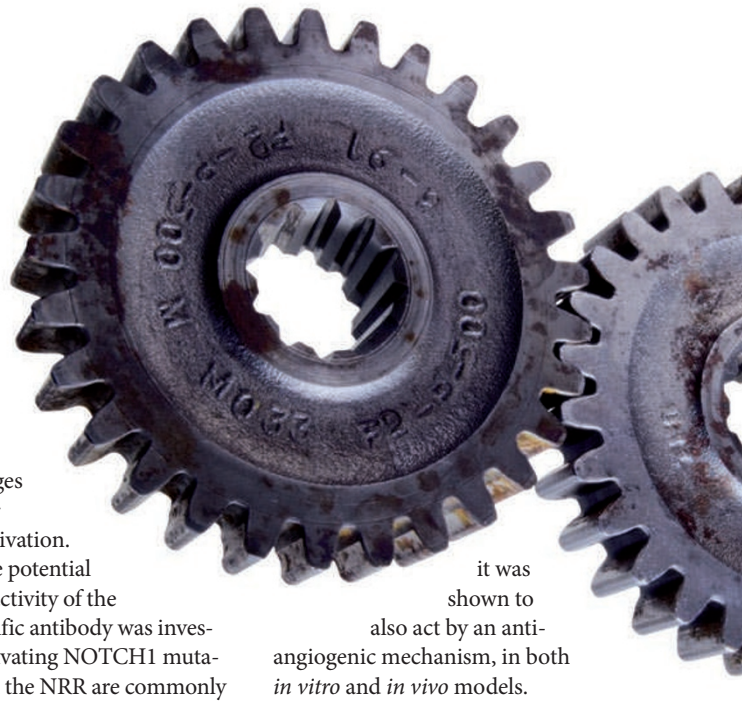
ANTICANCER DRUGS

A Notch closer



Aberrant signalling of the Notch pathway has been linked to numerous diseases, particularly many cancers. Although the potential of therapeutically targeting this pathway in cancer is being actively investigated, strategies used so far have been nonspecific or have been associated with significant side effects. Now, writing in *Nature*, Wu and colleagues characterize antibodies that selectively inhibit either NOTCH1 or NOTCH2 receptor signalling, exerting anticancer activity in mice without toxicity.

The Notch family of transmembrane receptors comprises four members, with roles in the regulation of cell fate and growth. Following ligand binding, a conformational change in the receptor negative regulatory region (NRR) occurs, which ultimately facilitates initiation of the downstream Notch transcriptional programme. The authors therefore proposed that targeting this vital step in Notch pathway signalling may represent a new therapeutic approach.

First, using phage display technology, the authors generated fully human immunoglobulin G1 NRR1-specific and NRR2-specific antibodies, which selectively inhibited NOTCH1 or NOTCH2 signalling. Receptor specificity was confirmed *in vivo* by the ability of the NRR1-specific and NRR2-specific antibodies to reduce the population of mouse T cells and splenic marginal zone B cells, respectively. Characterization of the crystal structure of the NRR1-specific antibody Fab fragment bound to human NRR1 suggested that the antibody mediates its inhibitory effects through interference with the conformational changes required for receptor activation.

Next, the potential anticancer activity of the NRR1-specific antibody was investigated. Activating NOTCH1 mutations within the NRR are commonly observed in patients with T-cell acute lymphoblastic leukaemia (T-ALL) and allow ligand-independent signalling. In a NOTCH1 mutant T-ALL cell line, the NRR1-specific antibody significantly induced cell cycle arrest and reduced cell proliferation. Moreover, it also decreased cell size and increased apoptosis — effects that correlated with NOTCH1 inhibition. In addition, signalling activated by two of the most common T-ALL mutations (L1594P and L1575P), one of the strongest activating mutations (I1681N), as well as a PEST domain truncation (representative of another common cluster of T-ALL mutations), were inhibited in cell models.

Screening a panel of 45 cancer cell lines for sensitivity to the NRR1-specific antibody also discovered activity in the MT-3 human colon cancer cell line, which was due to a single activating NOTCH1 mutation, A1702T. Moreover, in mouse xenograft T-ALL and colon cancer models, the antibody induced significant tumour regression and slowing of growth, respectively. Interestingly, it was shown to also act by an anti-angiogenic mechanism, in both *in vitro* and *in vivo* models.

Finally, they assessed whether their antibodies induced intestinal toxicity, as this has been associated with pan-Notch inhibitors. Mice treated with both the NRR1-specific and NRR2-specific antibodies rapidly lost weight, whereas mice treated with either antibody alone maintained weight. Although the combination of both antibodies induced severe intestinal crypt goblet cell metaplasia, no such effect was detected with the NRR2-specific antibody alone, and only a mild effect was observed with the NRR1-specific antibody.

It seems that Notch receptor-specific antibodies represent a significant advance over existing Notch pathway inhibitors, and may prove to be valuable in the future treatment of cancers, as well as other indications linked to dysregulated Notch signalling.

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ORIGINAL RESEARCH PAPER Wu, Y. *et al.* Therapeutic antibody targeting of individual Notch receptors. *Nature* **464**, 1052–1057 (2010)