tient gender and age. Consent to use these specimens in the current study was obtained from the relevant Local Research Ethics Committee. The sections were dewaxed and rehydrated, then probed for CYP1B1 using a commercially available antibody. Bound antibody was detected using a peroxidase/diaminobenzidine (DAB) visualising system. Antibody validity was confirmed by western blots using the same antibody, and by limited replication of the immunohistochemistry using a privately supplied antibody. Malignant epithelial cells were positive for CYP1B1 in over 75% of cases, as was normal colon epithelium. However, results showed that the staining was not confined to the malignant epithelium within tumours. The tumour-associated smooth muscle and blood vessel pericytes were positive in all our specimens. Macrophages were also identified as positive for CYP1B1. A number of colon samples, removed some distance from the tumour, reveal that expression of CYP1B1 in pericytes and colon epithelium is decreased. These preliminary results indicate upregulation of CYP1B1 in tumour-associated blood vessels. CYP1B1 is not confined to malignant epithelium. These data confirm that CYP1B1 is expressed in cells within colon tumours, particularly in pericytes surrounding tumour vasculature. The protein is almost undetectable in pericytes some distance from the tumour. Expression of this drug metabolising enzyme in tumours may have implications for carcinogenesis studies, as well as the metabolism of current and future chemotherapeutics.

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Elucidation of the molecular mechanism underlying the anti-invasive activity of a novel proteasome inhibitor (Bz-Leu-Leu-Leu-COCHO)

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The ubiquitin/proteasome pathway is an attractive chemotherapeutic target because of its involvement in fundamental cell processes such as cell cycle progression and transcriptional regulation. Particular interest in the proteasome has arisen since it was discovered to play a major role in controlling the activity of the oncogenic transcription factor NF-kB. We have synthesized a peptidyl alpha–keto aldehyde and shown it to be a reversible inhibitor of the chymotrypsin-like proteolytic activity of the proteasome (Ki=3.7nM). Interleukin-1 (IL-1) is a known activator of NF-kB and it stimulates the proteasomal degradation of the inhibitor of NF-kB, IkB, so that NF-kB can translocate into the nucleus and exert its effects. Assays looking at the ability of RKO and HT29 colorectal carcinoma cell lines to invade through an extra-cellular matrix mimic revealed that IL-1 (10 ng/ml) induced a 2-fold increase in the invasive capacity of these cell lines. In the presence of our proteasome inhibitor this could be reversed to levels below basal invasion in a dose dependent manner (100 nM- 10 uM). In HT29 cells the compound also inhibited unstimulated invasion of the cells but no effect on this basal invasion was observed in the RKO cell line. MMP-9 in conditioned medium from the two cell lines was detected by an enzyme linked immunsorbent assay (ELISA). Stimulation of the cells with IL-1 (10 ng/ml) increased the secretion of MMP-9 by approximately 2-fold and this could be reduced to sub-basal levels by treatment with our proteasome inhibitor. This effect on MMP-9 activity is in keeping with a previous report that has demonstrated the presence of a NF-kB response element in the gene of this protease. Luciferase assays have confirmed that IL-1 stimulates transcription of MMP-9 and further experiments are underway to determine whether the proteasome inhibitor reduces MMP-9 secretion at the transcriptional level. In summary these results provide strong evidence that inhibition of the ubiquitin/proteasome pathway represents a potential target for anti-cancer therapy. This compound represents a useful lead compound for this approach and the full role of NF-kB in this anti-invasive activity is under further investigation.