The role of CD44 in the trastuzumab resistance breast cancel cell line

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Activation of the ErbB family of receptor tyrosine kinases is involved in a range of human cancers. Transmembrane signaling mediated by ErbB proteins is stimulated by peptide growth factors and is blocked by monoclonal antibodies such as trastuzumab and pertuzumab. Trastuzumab is a recombinant, humanized anti-ErbB2 antibody, which is widely used for the treatment of breast cancer. Despite encouraging clinical results some cancers are primarily resistant to trastuzumab, and a majority of those initially responding become resistant during prolonged treatment. The mechanisms of trastuzumab resistance have not been fully understood but it is known that ErbB receptors exert their function in conjunction with non-ErbB proteins, e.g. CD44.

We examined the possible mechanisms of trastuzumab resistance, the role of masking of ErbB2 in connection with CD44 expression and synthesis of its ligand, hialuronan and significance of CD44 shedding. We used the breast cancer cell line JIMT-1 established from the pleural metastasis of a patient who was clinically resistant to trastuzumab. Despite ErbB2 gene amplification and receptor overexpression, JIMT-1 cells are resistant to trastuzumab *in vitro*, and also *in vivo*, if therapy is initiated 45 days after establishing xenografts.

We show that high expression of CD44 observed in JIMT-1 cells correlates with ErbB2 downregulation *in vivo*, while siRNA-mediated inhibition of CD44 expression leads to decreased rate of trastuzumab internalization and low cell proliferation *in vitro*. An inhibitor of hyaluronan synthesis, 4-methylumbelliferon (4-MU) significantly reduced the hialuronan level of JIMT-1 cells both *in vivo* and *in vitro* leading to enhanced binding of trastuzumab to ErbB2 and increased ErbB2 down-regulation. Furthermore, the inhibitory effect of trastuzumab on the growth of JIMT-1 xenografts was significantly increased by 4-MU treatment. Our results show that epidermal growth factor (EGF) and heregulin induce CD44 shedding in JIMT-1, an ErbB2-overexpressing cell line resistant to trastuzumab, accompanied by internalization and intramembrane proteolysis of CD44 and enhanced cellular motility. These effects of EGF and heregulin are blocked by pertuzumab. Trastuzumab inhibits the heregulin- and hyaluronan oligosaccharide-induced shedding and internalization of CD44 and their motogenic effect. Trastuzumab also blocks CD44 shedding from JIMT-1 xenograft tumors *in vivo*. At the same time the internalization rate of trastuzumab is increased by hyaluronan oligosaccharide treatment *in vitro*.

Our experiments point to an unexpected, but potentially important mechanism of action of ErbB receptor-targeted monoclonal antibodies used in the treatment of cancer and show the importance of the CD44-hyaluronan pathway in the escape of tumour cells from receptor-oriented therapy.