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Special Article

Monoclonal Antibody Therapy of Human Cancer: Taking the HER2 Protooncogene to the Clinic

H. MICHAEL SHEPARD,^{1,4} GAIL D. LEWIS,¹ JAY C. SARUP,¹ BRIAN M. FENDLY,¹ DANIEL MANEVAL,¹ JOYCE MORDENTI,¹ IRENE FIGARI,¹ CLAIRE E. KOTTS,¹ MICHAEL A. PALLADINO, JR.,¹ AXEL ULLRICH,² and DENNIS SLAMON³

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The HER2 protooncogene encodes a 185-kDa transmembrane protein (p185^{HER2}) with extensive homology to the epidermal growth factor (EGF) receptor. Clinical and experimental evidence supports a role for overexpression of the HER2 protooncogene in the progression of human breast, ovarian, and non-small cell lung carcinoma. These data also support the hypothesis that p185^{HER2} present on the surface of overexpressing tumor cells may be a good target for receptor-targeted therapeutics. The anti-p185^{HER2} murine monoclonal antibody (muMAB) 4D5 is one of over 100 monoclonals that was derived following immunization of mice with cells overexpressing p185^{HER2}. The monoclonal antibody is directed at the extracellular (ligand binding) domain of this receptor tyrosine kinase and presumably has its effect as a result of modulating receptor function. *In vitro* assays have shown that muMAB 4D5 can specifically inhibit the growth of tumor cells only when they overexpress the HER2 protooncogene. MuMAB 4D5 has also been shown to enhance the TNF- α sensitivity of breast tumor cells that overexpress this protooncogene. Relevant to its clinical application, muMAB 4D5 may enhance the sensitivity of p185^{HER2}-overexpressing tumor cells to cisplatin, a chemotherapeutic drug often used in the treatment of ovarian cancer. *In vivo* assays with a nude mouse model have shown that the monoclonal antibody can localize at

the tumor site and can inhibit the growth of human tumor xenografts which overexpress p185^{HER2}. Modulation of p185^{HER2} activity by muMAB 4D5 can therefore reverse many of the properties associated with tumor progression mediated by this putative growth factor receptor. Together with the demonstrated activity of muMAB 4D5 in nude mouse models, these results support the clinical application of muMAB 4D5 for therapy of human cancers characterized by the overexpression of p185^{HER2}.

KEY WORDS: HER2; *neu*; TNF- α ; monoclonal antibody therapy.

BACKGROUND: THE HER2 PROTOONCOGENE AND HUMAN CANCER

Cellular protooncogenes encode proteins that are thought to regulate normal cellular proliferation and differentiation. Alterations in their structure or amplification of their expression lead to abnormal cellular growth and have been associated with carcinogenesis (1-4). Protooncogenes were first identified by either of two approaches. First, molecular characterization of the genomes of transforming retroviruses showed that the genes responsible for the transforming ability of the virus in many cases were altered versions of genes found in the genomes of normal cells. The normal version is the protooncogene, which is altered by mutation to give rise to the oncogene. An example of such a gene pair is represented by the EGF receptor and the *v-erbB* gene product. The virally encoded *v-erbB* gene product has suffered truncation and other alter-

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ations that render it constitutively active and endow it with the ability to induce cellular transformation (5).

The second method for detecting cellular transforming genes that behave in a dominant fashion involves transfection of cellular DNA from tumor cells of various species into nontransformed target cells of a heterologous species. Most often this was done by transfection of human, avian, or rat DNAs into the murine NIH 3T3 cell line (1-5). Following several cycles of genomic DNA isolation and retransfection, the human or other species DNA was molecularly cloned from the murine background and subsequently characterized. In some cases, the same genes were isolated following transfection and cloning as those identified by the direct characterization of transforming viruses. In other cases, novel oncogenes were identified. An example of a novel oncogene identified by this transfection assay is the *neu* oncogene. It was discovered by Weinberg and colleagues in a transfection experiment in which the initial DNA was derived from a carcinogen-induced rat neuroblastoma (6,7). Characterization of the *neu* oncogene revealed that it had the structure of a growth factor receptor tyrosine kinase, had homology to the EGF receptor, and differed from its normal counterpart, the *neu* protooncogene, by an activating mutation in its transmembrane domain (8).

The association of the HER2 protooncogene with cancer was established by yet a third approach, that is, its association with human breast cancer. The HER2 protooncogene was first discovered in cDNA libraries by virtue of its homology with the EGF receptor, with which it shares structural similarities throughout (5). When radioactive probes derived from the cDNA sequence encoding p185^{HER2} were used to screen DNA samples derived from breast cancer patients, amplification of the HER2 protooncogene was observed in about 30% of patient samples (9). Further studies have confirmed this original observation and extended it to suggest an important correlation between HER2 protooncogene amplification and/or overexpression and worsened prognosis in ovarian cancer and non-small cell lung cancer (10-14).

The association of HER2 amplification/overexpression with aggressive malignancy, as described above, implies that it may have an important role in progression of human cancer; however, many tumor-related cell surface antigens have been described in the past, few of which appear to have a

direct role in the genesis or progression of disease (15,16). The data which support a role of HER2 overexpression in the basic mechanisms of human cancer are summarized below.

Amplified expression of p185^{HER2} can lead to cellular transformation as assessed by morphological alterations and growth of p185^{HER2}-overexpressing cells in soft agar and in nude mice (17,18). In addition, NIH 3T3 fibroblasts overexpressing p185^{HER2} have an increased resistance to cytotoxicity mediated by activated macrophages or recombinant human TNF- α (19), the cytokine that appears to be mainly responsible for macrophage-mediated tumor cell cytotoxicity (20). This observation extends also to breast tumor cells, which overexpress p185^{HER2} (19), and suggests that high levels of p185^{HER2} expression may be related to tumor cell resistance to at least one component of the host's antitumor surveillance armamentarium, the activated macrophage. This work has been reviewed previously (21), and similar data have recently been reported for ovarian tumor cell lines which overexpress p185^{HER2} (22). Further support for a role of p185^{HER2} or the related *neu* oncogene-encoded tyrosine kinase in tumorigenesis comes from work with transgenic mice that have been manipulated to overexpress one or the other of these two related genes. Transgenic mice expressing the activated form of the rat *neu* protooncogene, under the control of a steroid inducible promoter, uniformly develop mammary carcinoma (23). In another transgenic mouse model the HER2 protooncogene product, "activated" by point mutation analogous to the rat *neu* oncogene product, or an unaltered form of the HER2 protooncogene, has been expressed in mice (24). The main malignancies induced in this model were either lung adenocarcinoma or lymphoma but not mammary carcinoma. While it is not known why the different transgenic mouse models give such distinct results, the latter model may be of particular significance given the recent report of an association between p185^{HER2} overexpression and poor prognosis in nonsmall cell lung cancer (14). These differing results suggest some difference in the activity of activated *neu* and HER2-encoded tyrosine kinases, although effects due to mouse strain differences cannot be excluded.

The structural similarities between p185^{HER2} and the EGF receptor suggest that function of p185^{HER2} may be regulated similarly to the EGF receptor. In particular, one expects that the tyrosine kinase activity associated with the cytoplasmic domain of

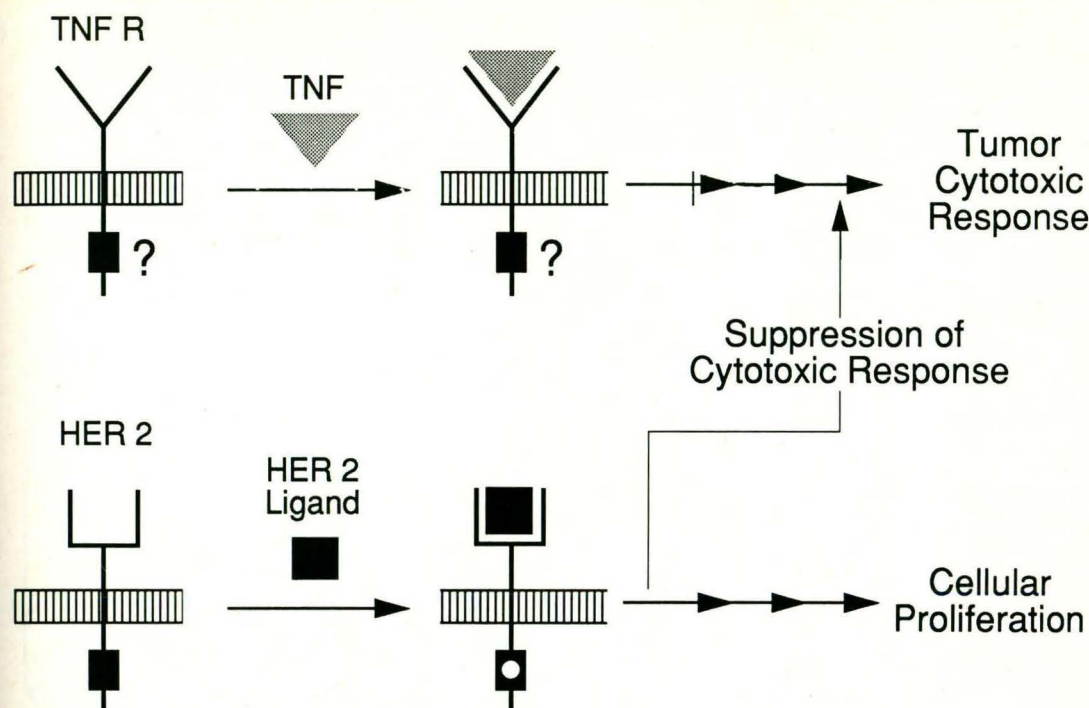


Fig. 1. Suppression of the TNF cytotoxic response by activation of p185^{HER2}. The schematic shows both the TNF cytotoxic pathway (top) and the p185^{HER2}-stimulated cell proliferation/transformation pathway (bottom). Signaling from the TNF receptor following interaction with TNF has not been characterized. Binding of ligand to p185^{HER2} is shown to activate the receptor-associated tyrosine kinase activity, resulting in stimulation of cellular proliferation and suppression of the tumor cell cytotoxic response to TNF.

the receptor would be ligand activated. This proposal receives support from recent work describing a ligand for p185^{HER2} (25). These data lead to a model (Fig. 1) wherein antagonists that downregulate the function of p185^{HER2} should have the effect of inhibiting growth of tumor cells dependent upon p185^{HER2} function and of increasing the sensitivity of such tumor cells to TNF- α . By analogy with previous work done with two related tyrosine kinases, the EGF receptor (26) and the activated *neu* protooncogene product (27), we hypothesized that monoclonal antibodies targeted to the extracellular domain of p185^{HER2} may have the desired properties.

DERIVATION OF muMAb 4D5

A family of monoclonal antibodies focused against the extracellular domain of p185^{HER2} were prepared (28). To do this, an NIH 3T3 fibroblast cell line that overexpresses p185^{HER2} [NIH 3T3/HER2-3-400 (18)] was used to immunize BALB/c mice. The mice were subsequently boosted with NIH 3T3/HER2-3-400 and, finally, with a preparation

enriched for p185^{HER2} by wheat germ agglutinin chromatography of membrane extracts of this cell line. Following splenocyte fusion with a mouse myeloma partner, the hybridomas were cultured in 96-well microtiter plates. Hybridomas positive for anti-p185^{HER2} activity, but with little or no anti-EGFR activity, were detected by ELISA (Fig. 2). A critical property of an anti-p185^{HER2} monoclonal antibody with potential for therapy would be its lack of cross-reactivity with the closely related EGF receptor, which is expressed at elevated levels in multiple tissues (29). To select further monoclonal antibodies with this characteristic, a number of assays were performed, including immunoprecipitation assays utilizing *in vivo* labeled EGF receptor and p185^{HER2} (Fig. 3A) and FACS analysis of antibody binding to tumor cells overexpressing either p185^{HER2} or the EGFR (Fig. 3B). The screening results are summarized in Table I. Based upon these results, nine of the p185^{HER2} monoclonal antibodies were chosen for further characterization, including a cell growth inhibition assay utilizing the SK-BR3 human breast adenocarcinoma cell line, which greatly overexpress p185^{HER2}. The monoclo-

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