Neoplasia • Vol. 4, No. 3, 2002, pp. 237–242 237
www.nature.com/neo

Antitumor Effect of MAb EMD 55900 Depends on EGF-R Expression and Histopathology[1]

Christine Solbach, Anja Sterner-Kock†, Marc Roller‡, Hans Georg Schnürch§, Manfred Stegmüller*, Gudrun Caspar-Bell¶, Petra Maria Schumm-Draeger¶, Manfred Kaufmann* and Rainald Knecht‡*

*Department of Gynecology and Obstetrics, †Central Research Unit, ‡Department of Otolaryngology, ¶Department of Medicine I, School of Medicine, Johann Wolfgang Goethe University, Frankfurt am Main, Germany; §Lukas Hospital, Neuss, Germany

Abstract

The proliferative stimulus of the epidermal growth factor (EGF) in human epithelial cells is mediated by its binding to the external domain of the EGF receptor (EGF-R). The purpose of this study was to investigate whether growth arrest of tumors treated with anti-EGF-R MAb (EMD 55900) was dependent on EGF-R expression and distinct histopathologic criteria of those neoplasms. Nine different adenocarcinomas, squamous cell carcinomas and two neoplastic epithelial cell lines (A431 and Detroit 562), which were characterized by high EGF-R expression, were xenotransplanted onto NMRI-nu/nu mice and treated with an anti-EGF-R antibody (EMD 55900). Results revealed that EGF-R expression and distinct histopathologic growth patterns play an important role for the therapeutic effect of the EGF-R antibody treatment. Tumors with high epithelial cellularity and little connective tissue responded to EMD 55900 treatment to a greater degree of growth reduction than tumors with lower cellularity. These results will be helpful for evaluation of patients who would benefit from tumor therapy with anti-EGF-R antibody.

Neoplasia (2002) **4,** 237–242 DOI: 10.1038/sj/neo/7900236

Keywords: EGFR, antibody, histology, EMD 55900, EMD 72000.

Introduction

The proliferative stimulus of cytokines like epidermal growth factor (EGF) is mediated by its binding to the extracellular domains of a specific transmembrane receptor family. These receptors are known as the c-erbB receptors, which consist of at least the four members c-erbB-1 to c-erbB-4. Binding of natural ligands like EGF or transforming growth factor alpha (TGF-α) results in dimerization of these receptors, whereby the formation of homo- and heterodimers is described (reviewed in Ref. [1]). Dimerization results in cross- and/or autophosphorylation processes, mediated by intracellular C-terminal tyrosine kinase residues. The phosphorylated and activated receptors are recognized by specific proteins, which in return activate a signaling cascade, the Ras–Raf–MEK kinase cascade, resulting in recruitment and activation of transcription factors [2-4]. However, erbB-2/erbB-3 heterodimers possess a special role in this context due to the fact that erbB-3 is kinase dead, has about six binding sites for PI-3 kinase, and erbB-2 has no high affinity ligand. The formation of these dimers is driven by neuregulins (NRG1-α/β and NRG2-α/β) as ligand [5,6].

Analyses of solid tumors revealed a correlation between the 170-kDa transmembrane EGF receptor (c-erbB-1, EGF-R) expression and histopathologic grading, whereby tumors with low EGF-R expression showed a higher degree of differentiation and correlate with an improved prognosis for the patients [7,8]. EGF-R protein overexpression is described for a variety of human malignancies, including breast cancer, ovarian cancer, prostate cancer, non–small cell lung cancer, head and neck cancer, and glioblastomas. These findings define the EGF-R as a valuable target for an antibody-mediated tumor therapy [9-11] and led to the development of the EGF-R antibody EMD 55900 [12]. This monoclonal antibody binds to the extracellular domain of EGF-R, close to the EGF binding domain, and does not induce any tyrosine kinase activity on its own whereby EMD 55900 binding inhibits receptor activation by natural ligands thereby interrupting activation of downstream signaling cascade activated by EGF or TGF-α, respectively [12,13].

This led to the perception that the therapeutic effect of EMD 55900 in human breast cancer is dependent on EGF-R protein expression levels of the tumor in general. In a previous study, we were able to show a dependency between EGF-R level and therapeutic effect of EMD 55900 for xenotransplanted breast cancer [14]. Actually, breast cancers are known to have a relative low level of EGF-R protein expression. In contrast, it was demonstrated

Address all correspondence to: Dr. Rainald Knecht, Department of Otolaryngology, School of Medicine, Johann Wolfgang Goethe University, Theodor-Stern Kai 7, D-60590 Frankfurt am Main, Germany. E-mail: knecht@em.uni-frankfurt.de

[1]This work was supported by Deutsche Forschungsgemeinschaft BE 1068/2-1 and E. Merck, Darmstadt.

Received 12 November 2001; Accepted 28 December 2001.

Copyright © 2002 Nature Publishing Group All rights reserved 1522-8002/02/$25.00

that squamous cell carcinomas and some adenocarcinomas of different origins showed a much higher content of EGF-R than breast cancer, whereby tumors of the cervix, vulva, ovaries, pharynx/larynx, and thyroid gland show up to tenfold higher EGF-R content than breast cancer [14-19]. The aim of this study was to demonstrate the therapeutic effect of EMD 55900 with respect to growth inhibition of human squamous cell carcinomas and adenocarcinomas with high EGF-R content and to find histopathologic criteria that may influence the therapeutic results.

Materials and Methods

Monoclonal Antibodies

EMD 55900 is a murine IgG2a directed against the human EGF-R and was first described by Murthy et al. [12]. The humanized version of this antibody (EMD 72000) was used for comparison.

All antibodies for this study were provided by E. Merck (Darmstadt, Germany).

Animal Experiments

Xenotransplantation and tumor measurement Tumor specimens of different origin of patients who were treated between 1976 and 1990 in the University Hospital of Frankfurt/Main, Germany were transplanted onto athymic nude mice and kept as xenotransplants. Nine human tumors and two solid specimens from a human vulva carcinoma cell line (A-431) and a human larynx cell line (Detroit 562) were transplanted subcutaneously as tissue fragments of 2mm^3 size onto 4- to 5-week-old nude mice. The tumor growth in nude mice was measured with vernier calipers weekly, and in case of A431, every 3 days. Tumor area was calculated by multiplication of the greatest diameter with the perpendicular diameter. Measurements were taken once a week. Measurements of all tumors within the group were represented by the mean value. Mean values (square millimeters) were plotted against time (days) post transplantation, resulting in growth curves.

Treatment Protocol From all 11 tumors the individual growth pattern of each tumor was examined in 14 to 21 animals, half of them treated with EMD 55900 and the others with phosphate-buffered saline (PBS; 0.15 M NaCl, 1.5 mM NaH_2PO_4, 5 mM Na_2HPO_4, pH 7.4) to serve as control group.

The treatment protocol started on day 0, which is the day of first tumor measurement, 1 week after tumor transplantation. According to our previous investigations [14] 100 mg/kg of EMD 55900 in 0.45 ml PBS was injected intraperitoneally into each mouse of the therapy group. The control group received 0.45 ml PBS only. To examine the therapeutic effect to tumors of larger diameters, groups of seven animals were treated on day 12 or day x (x means the time when a tumor size of about 70 mm^2 was reached) with 100 mg/kg EMD 55900 for the cervical cancer (CV2) and the ovarian cancer (OV2).

Histopathologic Examination

Tumors of the control group were carefully removed from the subcutis and weighed. Afterwards each tumor was cut in two parts, cystic fluid and necrosis were removed, and the tumor was weighed again. One half was deep-frozen for the EGF-R determination and the second half was fixed in 4% phosphate-buffered formalin, dehydrated, and embedded in paraffin. One section of each tumor was stained with hematoxylin/eosin and another section was stained according to Goldner [15] for examination of the connective tissue content and necrosis.

Immunohistochemical Staining of CD31

Angiogenesis was determined by means of immunohistochemical staining with CD31. Sections (3- to 4-μm) were deparaffinized and boiled five times for 3 minutes in a citric buffer (10 mM citric acid, 10 mM sodium citrate, pH 6.0). Rat anti-mouse CD31 (Clone nER-MP12, Dianova, Hamburg, Germany) was diluted 1:10 in PBS and the tissues were incubated for 1 hour at room temperature with primary antibody. After washing with PBS the tissues were stained with an anti-rat super sensitive AP-kit (Bio Genex (DCS), Hamburg, Germany) and Fast Red, blocked with levamisole (Bio Genex). Counterstaining was done with hematoxylin according to Mayer for 1 minute. The results are expressed in a semiquantitative fashion and with a score from 0 to 5, where 0 means no CD31 detectable and 1 to 5 little to strong staining.

EGF-R Quantitative Assay

Tissues obtained from excised tumors were dissected and pulverized in liquid nitrogen by a microdismembrator (B. Braun, Melsungen, Germany). The tissue powder was suspended in lysis buffer (0.14 M NaCl, 2.6 mM KCl, 8 mM Na_2HPO_4, 1.4 mM KH_2P0_4 pH 7.4, 1% Tween-20) then homogenized in a Teflon/glass homogenizer. Ultracentrifugation for 1 hour at 100,000g yielded a supernatant containing the cytosolic fraction and solubilized cell membranes. Protein content was determined in the supernatant by Bio-Rad protein assay (Biorad, Munich, Germany). The solution was normalized to a protein content of 50 μg/ml. From each sample, 200 μl in duplicate were used to measure EGF-R concentration by ELISA (Immundiagnostik, Bensheim, Germany).

Statistics

Mann–Whitney U tests were performed for statistical evaluation of significant differences in growth patterns between two study groups. Kruskal–Wallis test was used if more than two groups were compared. Probabilities were

Table 1. EGFR Content and Histological Criteria of the Tumor Specimens Used.

Region	Xenotransplant	Passage	Entity
Uterine cervix	CV1	VII	SCC
Uterine cervix	CV2	X	SCC
Uterine cervix	CV3	XII	SCC
Uterine cervix	CV4	IX	SCC
Ovarian	OV1	XI	Adenocarcinoma
Ovarian	OV2	XI	Adenocarcinoma
Vulva	VU1	IX	SCC
Breast	BC5	CVII	Adenocarcinoma
Thyroid	TH1	VL	Adenocarcinoma
Larynx	Detroit 562 (ATCC; No. CCL-138)	VII	SCC
Vulva	A431 (ATCC; No. CRL-1555)	V	SCC

considered significant at $P \leq 0.05$. Correlation coefficients were determined using Spearman-Rho analysis.

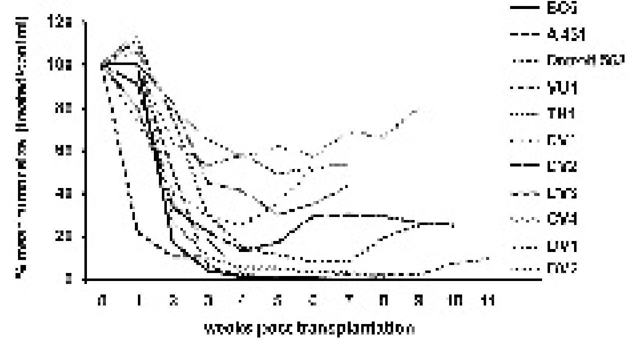

Figure 1. *Relative growth curves of treated tumors divided through control tumors from 11 xenotransplanted human tumors after single therapy with EMD 55900 (100 mg/kg i.p. on day of first tumor measurement [day 0]). The tumors A431, BC5, VU1, TH1, OV2, Detroit 562, and CV2 show a complete remission a few weeks following EMD 55900 application. The tumors OV1, CV1, and CV3 show a 50% growth reduction 4 to 8 weeks after antibody application whereas the tumor CV4 shows a growth reduction of 25% 9 weeks after EMD 55900 treatment.*

Results

Growth Inhibition of Xenotransplanted Tumors by EMD 55900

To determine the growth inhibitory effects of the monoclonal antibody EMD 55900 *in vivo*, we established 11 different tumors as xenotransplants in athymic nude mice. Nine tumors were obtained from spontaneously occurring primary carcinomas, whereby five tumors were primary squamous cell carcinomas of the uterine cervix and vulva and four were primary adenocarcinomas from breast, thyroid, and ovarian cancer (Table 1). The level of EGF-R protein expression was determined in excised control tumors for each experiment and ranged from 28 to 355 fmol/mg protein with a median EGF-R expression of 112 fmol/mg protein (Table 2). The animals were subjected to EMD 55900 antibody treatment at a single dose of 100 mg/kg body weight applied intraperitoneally 7 days post transplantation. All treated animals showed tumor growth inhibition by antibody treatment in comparison to the corresponding controls in the following observation period (Figure 1), whereby the level of significance ranged from $P=0.455$ to $P<0.0001$ (data reflected in Table 2). However, there was no significant correlation between increased EGF-R protein expression and an increased sensitivity to antibody treatment at the point of maximum growth reduction detectable.

Therapeutic Effect of EMD 55900 Correlates with Histopathologic Parameters

To further elucidate parameters that influence therapeutic effects of EMD 55900 we performed histopathologic examinations of control tumors. Tumors were excised at the end of the corresponding observation period and necrosis was described macroscopically. Furthermore, the tumors were weighed before and after dissection to determine the relative level of liquid proportion (Table 2). The content of connective tissue, degree of vascularization, and cellularity were determined microscopically. All tumors exhibited a distinct degree of central necrosis, whereby macroscopic features of those tumors (formation of cystic spaces, necrosis, connective tissue), as well as the degree of vascularization correlated with the relative proportion of connective tissue. The CD31 score/degree of connective tissue ratio (RT) revealed a factor that correlated with the therapeutic effect of EMD 55900 ($P=0.005$, $R=0.7$, Spearman-Rho analysis). Tumors with an RT$\leq$0.1 showed an inhibition of tumor

Table 2. Histological Criteria in Correlation to the Effects of Anti-EGFR Therapy.

Tumor	BC5	VU1	TH1	OV2	A431	Detroit 562	CV2	CV1	CV3	OV1	CV4
% Fluid (cysts)	32	27	13.5	0	39	16.5	0	0	0	0	0
% Necrosis	10–20	15	40	10	10	20	70	70	40	50–60	70–80
Description of necrosis	fluid	fluid	soft smeary fluid	medium soft smeary	fluid	smeary fluid	medium soft	soft	firm	gelatinous	firm
Connective tissue (%)	10	0	40	20–30	5	20	20	20	30	10–20	20–30
EGFR (fmol/mg)	266	187	105	44	355	112	124	212	75	28	108
CD31 score	1	0	3	3	0	2	4	3	4	3	4
Maximum tumor reduction (%)	99,5	99,5	99	99	96	92	87	79	70	51	44
P<	0.0001	0.0001	0.0001	0.001	0.0001	0.0031	0.0001	0.008	0.0015	0.0001	0.455
RT	0.1	0	0.075	0.075	0	0.1	0.2	0.15	0.13	0.2	0.16

Fluid = liquefactive necrosis; Firm = coagulative necrosis; Others = mixed forms.

growth of at least 92% (growth inhibition ranged from 99.5% to 92.0%) whereas tumors with an RT>0.1 showed a maximum growth inhibition of 87.0% (range: 44.0 to 87.0%) (P<0.001, Mann–Whitney U). Figure 2 shows representative histologic sections for two different carcinomas whereby the tumor BC5 had an RT of 0.1 and CV4 had an RT of 0.16. Furthermore, the RT is also a factor to determine the nature of necrotic tissue (i.e., liquefactive and coagulative necrosis), whereby an RT≤0.1 is correlated with the description of a more liquid form of necrosis. These results are reflected in growth characteristics of the squamous cell carcinomas VU1 and CV4. The carcinoma VU1 with an RT of 0.0 showed maximum growth inhibitory effect to EMD 55900 treatment in contrast to CV4 with an RT of 0.16. EGF-R protein expression of CV4 was comparable to VU1 but CV4 showed nearly no response to antibody treatment (Figure 3).

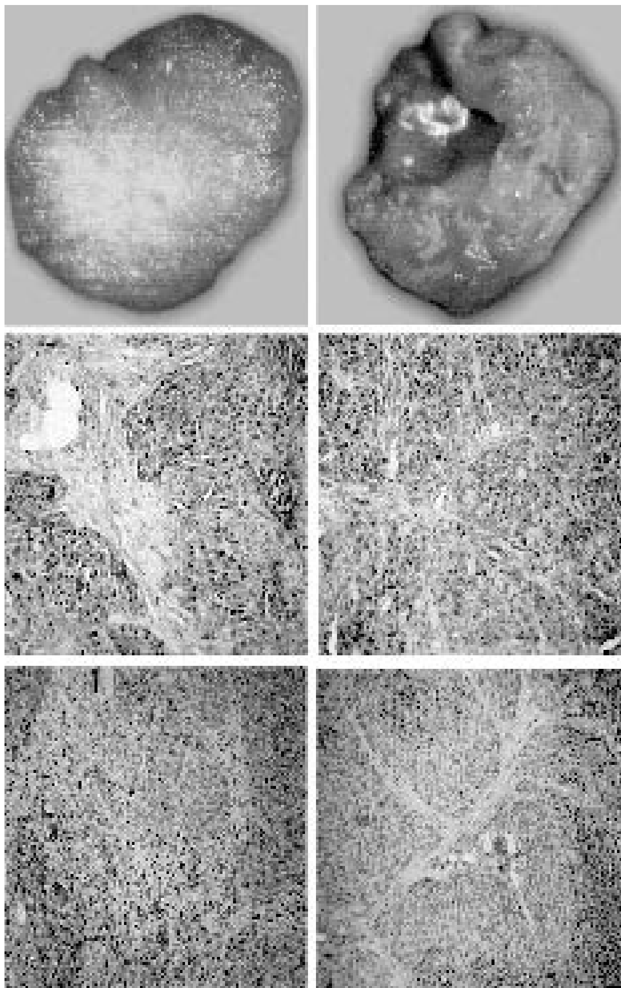

Figure 2. *Typical comparison of two tumors with different RTs. Image of the excised and sectioned tumor of xenotransplanted cervical cancer CV4 with an RT of 0.16 shows a large area of coagulative necrosis (upper left). Furthermore, CV4 shows a high degree of connective tissue in the corresponding Goldner staining (middle left) as well as a high degree of vascularization (lower left) in CD31 staining. In contrast, tumor BC5 (RT=0.1) shows a central cyst (drained, upper right), a low content of connective tissue (middle right), and a low grade of vascularization (lower right).*

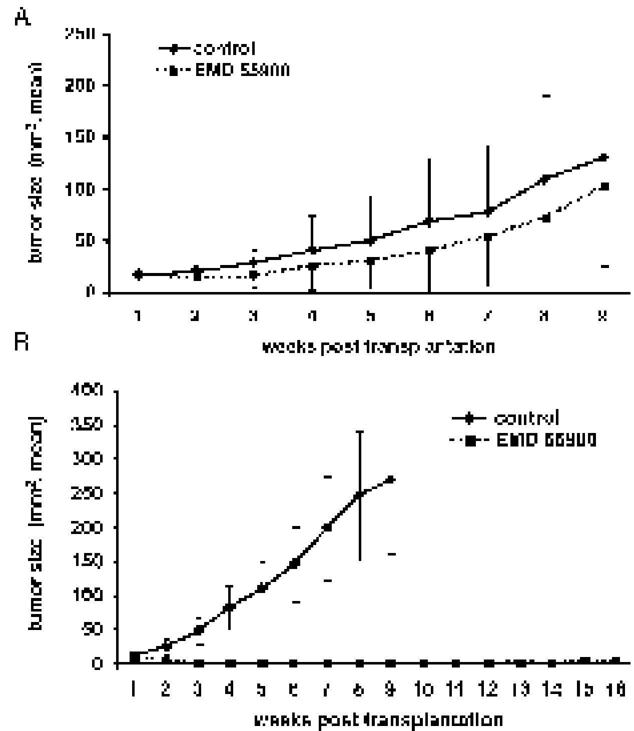

Figure 3. *(A) Growth curves of human cervical cancer CV4 with an EGF-R content of 108 fmol/mg protein transplanted onto nude mice and treated with a single dose of 100 mg/kg EMD 55900. The tumor is free of cysts, consists approximately 80% necrosis and 20% to 30% connective tissue (RT=0.16). Treatment with EMD 55900 revealed no significant growth reduction in comparison to the corresponding controls (P=0.455, n=16 for each group, Mann–Whitney U test). (B) Growth curve of human vulva cancer VU1 with an EGF-R content of 187 fmol/mg protein transplanted onto nude mice and treated with a single dose of 100 mg/kg EMD 55900. The tumor shows a cystic growth (27% of tumor mass is fluid), a very low content of connective tissue, and 5% necrosis (RT=0). The therapeutic effect is a complete remission 5 weeks after EMD 55900 treatment (P<0.0001, n=22 tumors in control group and n=20 tumors in EMD 55900–treated group, Mann–Whitney U test).*

To further determine the therapeutic effects of EMD 55900 on tumors of different sizes, we used two representative tumors that represented the two observed types of necrosis: CV2 (RT=0.2) and OV2 (RT=0.075). Three therapeutic groups were formed: The first group was treated on the day of first measurement (7 days post transplantation), the second was treated on day 12 after first measurement (mean tumor size was 20 mm^2 in both cases), and the third group was treated when mean tumor size reached 70 mm^2. Each group consisted of a minimum of 16 transplanted tumors and EMD 55900 antibody was given once at a constant dose of 100 mg/kg body weight. The results confirm the influence of RT at all stages of tumor establishment. The tumor OV2 with an improved prognostic RT of 0.075 showed increased susceptibility to EMD 55900 treatment and a prolonged period of tumor growth inhibition at all stages of tumor establishment. In contrast, the tumor CV2 showed no regression when tumor size exceeded 50 mm^2. Nevertheless, the duration of growth arrest seemed to be constant (for OV2 over 9 weeks and CV2 more than 5 weeks). After that period, in all

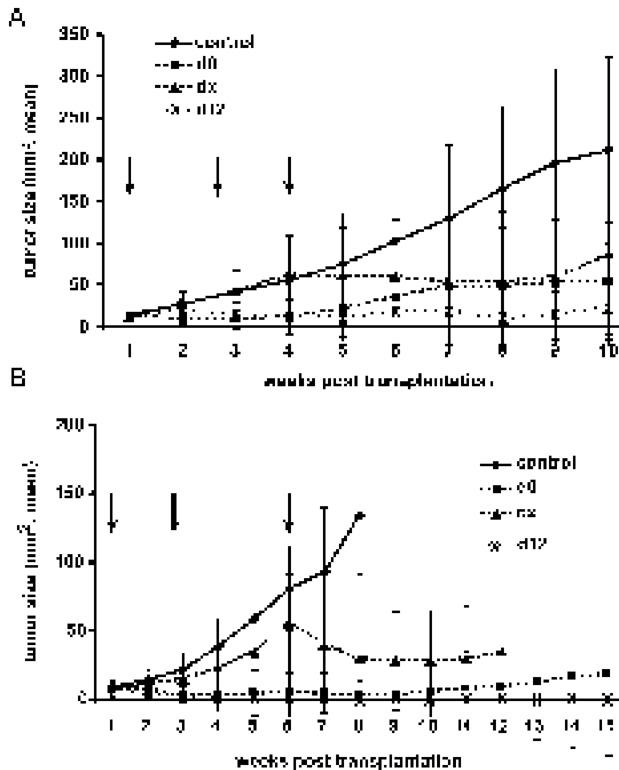

Figure 4. *(A) Growth curve of cervical cancer CV2 (RT=0.2) after single therapy with 100 mg/kg EMD 55900 on the day of first tumor measurement (d0), 12 days after first tumor measurement (d12), and when the tumor has reached a mean size of approximately 70 mm² (dx). Therapy on day 0 (d0) leads to an immediate stop of tumor growth for about 4 weeks followed by a remarkable slower tumor growth in comparison to the control. Therapy on day 12 causes a tumor regression for about 4 weeks and afterwards no detectable tumor growth for about 5 weeks. Therapy on day x (dx), in this case 4 weeks after transplantation, leads to a stop of proliferation for more than 5 weeks. The control group, dx and d12, represent 14 tumors, therapeutic group (do) represents n=16 tumors. (B) Growth curve of ovarian cancer OV2 (RT=0.075) after single therapy with 100 mg/kg EMD 55900 on the day of first tumor measurement (d0), 12 days after first tumor measurement (d12) and when the tumor has reached a mean size of approximately 70 mm² (dx). Therapy on day 0 (d0) leads to an immediate stop of tumor growth for about 10 weeks followed by a remarkable slower tumor growth in comparison to the control. Therapy on day 12 (d12) causes complete tumor regression for 15 weeks. Therapy on day x (dx), in this case 6 weeks after transplantation, leads to a tumor regression of about 50% for 7 weeks. The control group represents n=18 tumors, d0 and d12 therapeutic groups represent n=16 tumors, and dx therapeutic group represents n=12 tumors.*

cases a low grade of tumor growth progression was detectable (Figure 4).

Discussion

Growth factors and their receptors play an important role in regulating the growth of malignant cells *in vivo*. Several investigators have reported that overexpression of EGF-R in a variety of tumors is associated with poor prognosis for the patients [16,17]. The inhibitory effect of several anti–EGF-R antibodies such as MAb C225 on tumor growth have been extensively investigated in other tumor models. It has been shown that treatment of cells with these MAbs leads to inhibition of EGF-R downstream signaling, resulting in accumulation of cells in G1 due to upregulation of p27^{KIP-1} and hypophosphorylation of retinoblastoma protein pathway [18,19]. Treatment of a renal cell carcinoma cell line RCC with MAb C225 *in vitro* showed a weaker effect than *in vivo* xenograft model [20]. This supported the notion that antitumor effect in mice is not only a blockade of endogenous EGF. There might be an active influence of the immune system of the mouse [21]. Among direct effects of tumor-cell response, a variety of mechanisms of action of EMD 55900 are discussed, such as antibody-dependent cellular cytotoxicity (ADCC) by linking natural killer cells via Fc fragment of the antibody and phagocytosis by macrophages [21]. Furthermore, recent studies demonstrated also the down-regulation of secretion of neoangiogenic factors in response to anti-EGF-R antibody treatment [22,23]. This might also be a further mechanism *in vivo*, whereby EMD 55900 inhibits neovascularization in tumors exhibiting low pretreatment CD-31 score.

The present study was undertaken to assess the ability of the anti–EGF-R monoclonal antibody EMD 55900 to inhibit the growth of several human tumors with different EGF-R protein expression *in vivo*. The results of this study demonstrate that treatment with EMD 55900 inhibits the growth of larynx/pharynx, thyroid, breast, vulva, ovarian, and cervix carcinomas in athymic nude mice. Furthermore, we were able to demonstrate that the *in vivo* tumor response to EMD 55900 treatment is not only dependent of EGF-R protein expression.

We found the CD31/connective tissue ratio is a crucial factor for the prediction of a therapeutic effect of EMD 55900 in tumors with high EGF-R expression. Interestingly, we found that tumors exhibiting liquefactive forms of necrosis were more susceptible to antibody therapy. In contrast, tumors showing coagulative forms of necrosis were more resistant to the treatment. Our previous investigations found a strong correlation of EGF-R protein expression and response to EMD 55900 treatment of breast cancer, ranging from no effect at EGF-R-negative tumors to complete remission of tumors highly overexpressing EGF-R [14]. In contrast to this previous study, we investigated only EGF-R-overexpressing tumors and found that the cellular pattern and its histogenetic nature was significant in terms of tumor treatment. The precise mechanism of these findings remains to be further elucidated. Potentially, a reason for less response in stroma-rich tumors might be a paracrine stimulation of non–EGF-R growth factor systems like HRG-ErbB2/3, FGF, and PDGF [24,25]. We demonstrated that cervical, ovarian, breast, larynx/pharynx, and thyroid carcinomas that express functional EGF-R are responsive to antiproliferative activity of EMD 55900. Specific histopathologic features seemed of importance in this context, whereby highly cellular tumors seemed to be most responsive. The treatment with EMD 55900 has been documented to be a promising approach for the treatment of certain cancers. Additionally, CD31/connective tissue ratio could be a promising tool to improve the prediction of therapeutic value by monoclonal antibody therapy.

Acknowledgements

We thank R. Strohmeier for helpful comments on this manuscript, K. Frank and A. Greiner for excellent technical assistance.

References

[1] Earp HS, Dawson TL, Li X, and Yu H (1995). Heterodimerization and functional interaction between EGF receptor family members: a new signaling paradigm with implications for breast cancer research. *Breast Cancer Res Treat* **35**, 115–32.

[2] Yarden Y, and Sliwkowski MX (2001). Untangling the ErbB signaling network. *Nat Rev Mol Cell Biol* **2**, 127–37.

[3] Carraway LD, and Sweeney C (2001). Localization and modulation of ErbB receptor tyrosine kinases. *Curr Opin Cell Biol* **13**, 125–30.

[4] Wells A (1999). EGF receptor. *Int J Biochem Cell Biol* **31**, 637–43.

[5] Klapper LN, Glathe S, Vaisman N, Hynes NE, Andrews GC, Sela M, and Yarden Y (1999). The erbB-2/Her2 oncoprotein of human carcinomas may function solely as shared coreceptor for multiple stroma-derived growth factors. *Proc Natl Acad Sci USA* **96**, 4995–5000.

[6] Harari D, and Yarden Y (2000). Molecular mechanisms underlying ErbB2/HER2 action in breast cancer. *Oncogene* **19**, 6102–14.

[7] Pinkas-Kramarski R, Alroy I, and Yarden Y (1997). ErbB oncogenes and EGF-like ligands: cell lineage determination and oncogenesis through combinatorial signaling. *J Mammary Gland Biol Neoplasia* **2**, 97–107.

[8] Issing WJ, Wustrow TP, and Heppt WJ (1993). Oncogenes related to head and neck cancer. *Anticancer Res* **13**, 2541–51.

[9] Veale D, Ashcroft T, Marsh C, Gibson GJ, and Harris AL (1987). Epidermal growth factor receptors in non–small cell lung cancer. *Br J Cancer* **55**, 513–16.

[10] Drebin JA, Link VC, and Greene MI (1988). Monoclonal antibodies specific for the neu oncogene product directly mediate anti-tumor effects *in vivo*. *Oncogene* **2**, 387–94.

[11] Mendelsohn J, and Baselga J (2000). The EGF receptor family as targets for cancer therapy. *Oncogene* **19**, 6550–65.

[12] Murthy U, Basu A, Rodeck U, Herlyn M, Ross AH, and Das M (1987). Binding of an antagonistic monoclonal antibody to an intact and fragmented EGF-receptor polypeptide. *Arch Biochem Biophys* **252**, 549–60.

[13] Rodeck U, Williams N, Murthy U, and Herlyn M (1990). Monoclonal antibody 425 inhibits growth stimulation of carcinoma cells by exogenous EGF and tumor-derived EGF/TGF-alpha. *J Cell Biochem* **44**, 69–79.

[14] Schnürch HG, Stegmüller M, Vering A, Beckmann MW, and Bender HG (1994). Growth inhibition of xenotransplanted human carcinoma by a monoclonal antibody directed against the epidermal growth factor receptor. *Eur J Cancer* **4**, 491–96.

[15] Kiernann JA (1990). Histological and Histochemical Methods: Theory and Practice, 2nd ed. Pergamon Press, Oxford.

[16] Grandis JR, Melhem MF, Gooding WE, Day R, Holst VA, Wagener MM, Drenning SD, and Tweardy DJ (1998). Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. *J Natl Cancer Inst* **90**, 824–32.

[17] Nicholson S, Sainsbury JR, Halcrow P, Farndon JR, and Harris AL (1989). Expression of epidermal growth factor receptors associated with lack of response to endocrine therapy in recurrent breast cancer. *Lancet* **1**, 182–85.

[18] Busse D, Doughty RS, Ramsey TT, Russell WE, Price JO, Flanagan WM, Shawver LK, and Arteaga CL (2000). Reversible G(1) arrest induced by inhibition of the epidermal growth factor receptor tyrosine kinase requires up-regulation of $p27^{(KIP1)}$ independent of MAPK activity. *J Biol Chem* **275**, 6987–95.

[19] Fan Z, Shang BY, Lu Y, Chou JL, and Mendelsohn J (1997). Reciprocal changes in p27(Kip1) and p21(Cip1) in growth inhibition mediated by blockade or overstimulation of epidermal growth factor receptors. *Clin Cancer Res* **3**, 1943–48.

[20] Goldstein NI, Prewett M, Zuklys K, Rockwell P, and Mendelsohn J (1995). Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin Cancer Res* **1**, 1311–18.

[21] Houghton AN, and Scheinberg DA (2000). Monoclonal antibody therapies — a "constant" threat to cancer. *Nature* **4**, 373–74.

[22] Perrotte P, Matsumoto T, Inoue K, Kuniyasu H, Eve BY, Hicklin DJ, Radinsky R, and Dinney CPN (1999). Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing ortotopically in nude mice. *Clin Cancer Res* **5**, 257–64.

[23] Ciardiello F, Caputo R, Bianco R, Damiano V, Fontanini E, Cuccato S, De Placido S, Bianco AR, and Tortora G (2001). Inhibition of growth factor production and angiogenesis in human cancer cells by ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor. *Clin Cancer Res* **7**, 1459–65.

[24] Valverius EM, Ciardiello F, Heldin NE, Blondel B, Merlo G, Smith G, Stampfer MR, Lippmann ME, Dickson RB, and Salomon DS (1990). Stromal influences on transformation of human mammary epithelial cells overexpressing c-myc and SV40T. *J Cell Physiol* **145**, 207–16.

[25] Xie J, Aszterbaum M, Zhang X, Bonifas JM, Zachary C, Epstein E, and McCormick F (2001). A role of PDGFR alpha in basal cell carcinoma proliferation. *Proc Natl Acad Sci USA* **98**, 9255–59.