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ior egf/r3

Antineoplastic
Monoclonal Antibody

IOR-R3
mR3

Murine IgG_{2a} monoclonal antibody against the epidermal growth factor receptor (EGF-R)

EN: 210027

Introduction

The epidermal growth factor (EGF) and its receptor (EGF-R) have been intensively investigated in biological research during the last few years. The human EGF-R is a transmembrane glycoprotein which contains an extracellular EGF-binding domain, a transmembrane domain and a cytoplasmic domain, and has tyrosine kinase activity and undergoes autophosphorylation on ligand binding (1, 2). The EGF-R plays a key role in growth regulation of many animal cells by mediating the transmission of a signal across the plasma membrane. This agonist-regulated enzyme is a member of the family of receptor tyrosine kinases, each of which contains intrinsic protein tyrosine kinase activity necessary for transmission of the signal (3).

The overexpression of cell-surface growth factor receptors in human disease has been exploited in recent years to develop novel therapeutic agents that target the diseased cell. Reports of increased EGF-R expression in epithelial cancers suggest that aberrant expression of EGF-R and/or related genes may be involved in the pathogenesis of certain epithelial neoplasms (4-9). A relationship between tumor invasiveness and EGF-R expression has been observed in breast and bladder carcinomas (7, 8, 10) and enhanced EGF-R expression has been reported in human epidermoid lung tumors. High levels of EGF-R have also been identified in some gliomas, gynecological tumors and bladder tumors.

Although normal cells express EGF-R, the elevated number of receptors on tumor cells confers a degree of targeting specificity in that the tumor cells can proportionately bind more radiolabeled antibody. The monoclonal antibody (MAb) ior egf/r3, developed at the Center of Molecular Immunology in Havana, Cuba, is a murine IgG_{2a} antibody that recognizes the EGF-R. Its properties have been previously described (11, 12).

Description

The epidermal growth factor receptor (EGF-R) is a commonly expressed transmembrane glycoprotein that was the first of a number of receptors and growth factors found to be controlled by the proto-oncogene c-erb-B (13). The EGF-R is a single-chain glycoprotein of 1186 amino acids and has a molecular weight of 170 kDa. The EGF-R is composed of three major structural domains: an extracellular N-terminal ligand-binding domain which has 621 amino acids, a single hydrophobic transmembrane domain comprised of 23 amino acids and a 542-amino acid cytoplasmic intracellular C-terminal (14, 15) containing, among other regions, a protein tyrosine kinase domain. The extracellular N-terminal is divided into four domains; the ligand-binding site of the receptor is on the external surface of the plasma membrane on domains 1 and 3, whereas domains 2 and 4 are structural in nature and are enriched in cysteine amino acid residues (Fig. 1). The intracellular portion is a tyrosine kinase that is activated after binding of EGF or transforming growth factor α (TGFα) to the external domain of the receptor. The EGF-R can be autophosphorylated at Tyr^{1068}, Tyr^{1086}, Tyr^{1148} and Tyr^{1173}, resulting in receptor desensitization. The membrane receptor of EGF plays an important role in the transduction of the signals mediated by the growth factor. After binding, EGF and/or TGFα induce a broad range of early and delayed biologic responses leading to DNA synthesis in the target cell.

The EGF-R is one of a group of growth factor receptors that possess intrinsic tyrosine kinase activity and sequence homology to known cellular proto-oncogenes (16). The EGF-R tyrosine kinase mediates the mitogenic signal of EGF/TGFα and also determines the pathways whereby the ligand-receptor complex is internalized by the cell and transported to the lysosome where it is degraded; however, internalization itself does not require tyrosine kinase activity (13). This process, called downregulation, is one of the mechanisms by which

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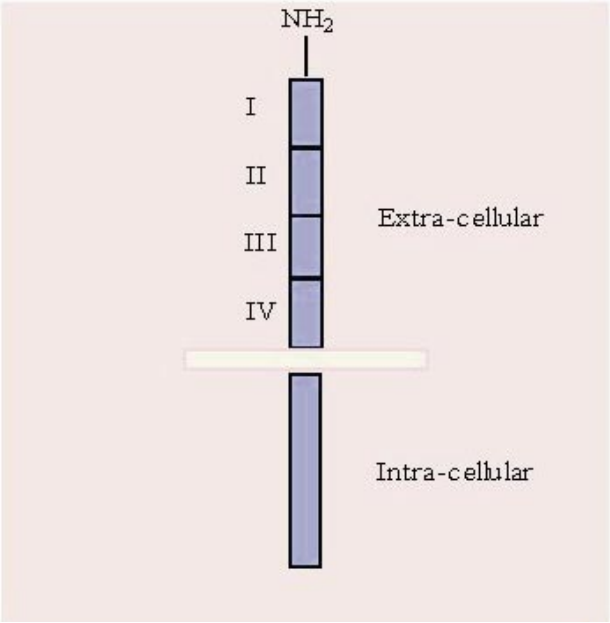

Fig. 1. Schematic structure of epidermal growth factor receptor, with the extracellular and intracellular domains.

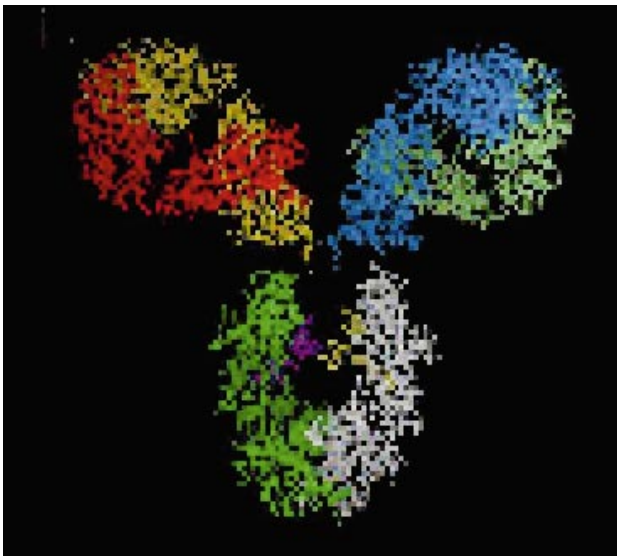

Fig. 2. Structural representation of ior egf/r3.

receptor activation is regulated. Signal transduction is accomplished through the tyrosine kinase by receptor oligomerization (13), by ligand-dependent autophosphorylation at three major sites (tyrosine residues numbers 1068, 1148 and 1173) (17) and by phosphorylation of exogenous substrates. Autophosphorylation is thought to be necessary for efficient phosphorylation of a number of exogenous substrates including p185neu, phospholipase C-γ, etc.

The involvement of EGF-R autocrine signalling pathways in the maintenance of tumor growth, particularly in progressive hormone-insensitive cancers such as breast and prostate, indicates that EGF-R represents a potential target for antineoplastic therapy. While some studies have investigated antibody-directed therapy which may inhibit growth by blocking receptor-ligand binding (18), another approach is to inhibit the activity of the EGF-R tyrosine kinase (EGF-RTK).

MAb ior egf/r3 is a highly specific murine monoclonal antibody which recognizes EGF-R (Fig. 2). It is secreted by hybridoma A24/15/128 obtained by fusion of murine myeloma cells SP2/Ag14 with splenocytes from Balb/c mice immunized with a partial purified fraction of the EGF-R from human placenta. Its generation and characterization (Table I) have been described in detail elsewhere (11, 12).

Table I: Structural and functional characteristics of ior egf/r3.

Parameter	ior egf/r3
Immunoglobulin isotype	IgG_{2a}
Molecular weight	150 kDa
Origin	Murine
Isoelectric point	6.48-7.22
Glycosilation pattern	G1
Affinity constant	0.94 ± 0.12 (10^8 l/mol)

Pharmacological Actions

MAb ior egf/r3, an IgG_{2a} isotype antibody generated against the external domain of the EGF-R, has several pharmacological properties. It blocks the binding of EGF or TGFα to the receptor, inhibits activation of receptor tyrosine kinase induced by EGF or TGFα binding, stimulates receptor internalization and inhibits cell proliferation induced by EGF or TGFα in *in vitro* cell culture and in nude mouse xenograft models.

In vitro *receptor binding*

Characterization of the ior egf/r3 MAb was performed through displacement experiments with the natural EGF ligand in a A431 cell line (a tumor cell line which overexpresses EGF-R), as well as in normal human placental tissue, which is also rich in the molecule (19). The dissociation constant (K_D) values obtained were in the range of 1-10 nM, which demonstrates the high affinity of the MAb for the receptor (11).

An antagonistic biological effect against EGF was demonstrated in experiments with the tumor cell line A431 when cultured with the EGF and ior egf/r3 at the

Table II: Pharmacokinetics of ior egf/r3 in experimental animal models.

Dose (mg)	Animal (No.)	Label	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)
1.0	Rats (45)	^{99m}TC	5.16 ± 1.12	20.06 ± 0.85
3.0	Monkeys (3)	^{188}Re	6.60 ± 2.13	25.40 ± 2.50

same time. The MAb was capable of inhibiting the effect of EGF in cell culture (19).

Receptor-positive MDA-468 breast cancer cell line demonstrated binding of ^{99m}Tc-labeled ior egf/r3, which could be blocked with 500 nM of native (unlabeled) MAb.

In vitro studies using H-125 human lung adenocarcinoma cell line and ior egf/r3 revealed that treatment with this EGF-R blocking antibody induced terminal differentiation.

Tumor tissue studies

Immunohistochemistry studies of recognition of tumor tissues by ior egf/r3 have shown differential staining intensity and distribution of the MAb in different types of tumors, with dependence on the expression level of the receptor. The tumors studied included gliomas, meningiomas, malignant fibrous histiocytoma, neurofibrosarcoma, breast, head and neck, and gynecological tumors. Results showed that ior egf/r3 specifically recognized all tumors of epithelial origin, but with different degrees of intensity. Nonepithelial tumors did not show any reactivity with the MAb (12,19).

The use of EGF-R blocking antibodies has been investigated using xenografts of a human lung adenocarcinoma cell line overexpressing EGF-R (20, 21). In these studies, ior egf/r3 was found to be uniformly localized on the tumor membranes and induced almost complete regression.

Pharmacokinetics in animals

Results of the pharmacokinetics of intact ior egf/r3 in experimental animals are shown in Table II. After intravenous administration, plasma time-activity curve of ^{99m}Tc-labeled ior egf/r3 best fit to biexponential functions with a distribution half-life ($t_{1/2\alpha}$) of 5.16 ± 1.12 h and an elimination half-life ($t_{1/2\beta}$) of 20.06 ± 0.85 h. The plasma time-activity curve for ^{188}Re-labeled ior egf/r3 was also biexponential, with a $t_{1/2\alpha}$ of 3.63 ± 2.93 h and a $t_{1/2\beta}$ of 25.4 ± 2.50 h.

Toxicology

Single-dose toxicity studies consisted of a single intravenous administration of ior egf/r3 at doses of 0.34, 3.4 and 8.5 mg/kg in female and male Sprague Dawley rats. No clinical signs of toxicity or abnormalities on autopsy of the animals were recorded.

A 14-day, repeated-dose toxicity study in male Sprague Dawley rats using the intravenous route was carried out with ior egf/r3 administered at dose levels of 0.34, 3.4 and 10.2 mg/kg/day (doses 30 times higher than the clinical dose). There were no significant signs of toxicity attributable to any dose of the MAb.

No conspicuous drug-related systemic or local toxicity at the injection site was seen in rats following administration of single and multiple i.v doses of up to 12.5 mg/kg.

Clinical Studies

In human clinical trials, ^{99m}Tc-labeled ior egf/r3 (1 and 3 mg) was administered as an i.v. bolus to 8 cancer patients to predict the best dosage and schedule for future clinical evaluations, to determine the pharmacokinetic parameters and to evaluate the differences between the two doses (23-27). Plasma time-activity curves in patients with tumor of epithelial origin were best fitted to a biexponential equation with a correlation coefficient of 0.98 ± 0.02 and with distribution half-lives ($t_{1/2\alpha}$) of 0.09 ± 0.03 h and 0.21 ± 0.06 h for 1 and 3 mg, respectively. Respective elimination half-lives ($t_{1/2\beta}$) were 25.1 ± 7.2 h and 13.9 ± 2.2 h for 1 and 3 mg (Table III). In the 5 patients administered 1 mg of the MAb, the shorter $t_{1/2\alpha}$ reflects the initial clearance from the central compartment and illustrates that the radiopharmaceutical was rapidly distributed to other compartments (tissues) outside the plasma. We believe this is due to rapid antibody-antigen binding between ^{99m}Tc-labeled ior egf/r3 and the EGF-R. The relatively longer elimination half-life reflects the retention of the radiopharmaceutical in the peripheral compartment (tumor and liver).

An estimation of the absorbed radiation doses to normal organs after i.v. administration of ^{99m}Tc-labeled ior egf/r3 was performed in patients with tumors of epithelial origin (23). The doses absorbed by an adult after administration of the MAb are presented in Table IV. For this radiopharmaceutical, the effective dose equivalent resulting from an administered radioactivity of 1470 MBq is typically 176.4 mSv (per 70 kg individual). For an administered radioactivity of 1470 MBq, typical radiation doses to the liver, spleen and kidneys are 1014.3, 543.9 and 120.5 mGy, respectively.

A multicenter study evaluating the safety and diagnostic efficacy of ^{99m}Tc-labeled ior egf/r3 was carried out

Table III: Pharmacokinetics of ^{99m}Tc-labeled ior egf/r3.

Dose (mg)	Tumor	No. patients	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)
1	Epithelial	5	0.09 ± 0.03	25.1 ± 7.2
3	Epithelial	3	0.21 ± 0.06	13.9 ± 2.2

Table IV: Internal radiation absorbed doses to normal organs after i.v. administration of ^{99m}Tc-labeled ior egf/r3.

Organ	Uptake (%)*	Absorbed dose (mGy/MBq)
Brain	0.04	0.010
Liver	41.20	0.690
Spleen	0.70	0.370
Kidneys	1.20	0.082
Rest of the body	60.20	0.049

*Uptake calculated by whole body images at 24 h after the administration of the radiopharmaceutical

in 148 patients (51 female and 97 male) with tumors of epithelial origin. Patients were injected with 3 mg of ior egf/r3 and 1470-1850 MBq of ^{99m}Tc. Overall sensitivity and accuracy of the immunoscintigraphic imaging were 84.2% (106/126) and 86.5% (128/148). New metastases not identified previously by other diagnostic methods were detected in 50% of the patients.

Conclusions

The overexpression of EGF-R in many tumors compared to normal tissue makes it possible to use the receptor as a target for diagnosis and follow-up of patients with tumors of epithelial origin and for the delivery of cytotoxins or radioactive isotopes preferentially to the tumor cells, as well as its use as a target for gene therapy.

EGF-R blocking antibodies have been tested in a number of phase I/II trials in patients with tumors of epithelial origin (22-28). These studies have shown that ior egf/r3 does not appear to cause adverse clinical reactions following its intravenous administration, that the MAb binds specifically to the tumor and that EGF-R blocking antibodies can be administered safely to patients having tumors overexpressing EGF-R. This suggests that the EGF-R plays an important role in the development of the malignant phenotype of many cancers.

Because overexpression of the EGF-R has been demonstrated in numerous solid human tumors and is associated with increased metastatic potential and poor prognosis, the receptor is a particularly attractive therapeutic target (29).

The major direction of research for improvement of diagnosis and therapy of malignant diseases with MAbs is in the field of genetic engineering. Genetic engineering will permit the development of "humanized" antibodies with biologic properties that favor tumor localization and treatment. Humanized antibodies, currently being used for diagnostic purposes, are being studied as potential therapeutic agents either naked or coupled to immunotoxins or radioisotopes for cancer treatment (30).

Source

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