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Interactions Between Growth Factor Receptors and Corresponding Monoclonal Antibodies in Human Tumors

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Monoclonal antibodies (MAbs) to the human epidermal growth factor (EGF) receptor, the type I insulin-like growth factor (IGF) receptor, and the nerve growth factor (NGF) receptor were used to study the growth regulation of malignant cells. Anti-EGF receptor MAb 425 inhibited the growth of A 431 squamous carcinoma cells which express high numbers of EGF receptors on their surfaces. Growth inhibition induced by MAb 425 was accompanied by alterations of the cell-cycle distribution of these cells, indicating the ability of a monoclonal antibody to act as a biologically active ligand. Growth stimulation of melanoma cells by EGF was unrelated to EGF receptor expression on the cell surface. Insulin- and IGF-I-induced growth stimulation of melanoma cells was inhibited by MAb αIR-3 which reacts with the type I IGF receptor. This result indicates that the type I IGF receptor mediated growth stimulation not only by IGF-I but also by insulin. Normal melanocytes and cells of all stages of tumor progression expressed in tissue culture the receptor for NGF, but no effect on the growth of these cells has been observed.

Key words: cancer, growth regulation

Recent advances in the purification and the genetically engineered production of polypeptide growth factors have facilitated studies on the role of these factors in growth regulation of mammalian cells. For most of the known growth factors, receptors expressed on the cell surface of responding cells have been identified. Monoclonal antibodies (MAbs) binding to growth factor receptors provide valuable tools to study effects of growth factors in malignant cells and their normal counterparts. Here, we report on the utilization of MAbs to three growth factor receptors for studies on the growth regulation of malignant cells.

Abbreviations used: EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; NGF, nerve growth factor; MAb, monoclonal antibody; MHA, mixed hemadsorption assay; RIA, radioimmunoassay; SRBC, sheep red blood cells; TGF, transforming growth factor.

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MATERIALS AND METHODS

MAb Binding Assay

Binding of MAbs to target cell surfaces in mixed hemadsorption assay (MHA) was determined using an indicator system consisting of sheep red blood cells (SRBC) precoated with mouse anti-SRBC antiserum and goat-antimouse immunoglobulin antiserum [1]. Positive cells were covered (>25% of perimeter) by rosettes of indicator cells. Binding of either ^{125}I-labeled MAb or ^{125}I-labeled growth factor to target cells was determined in radioimmunoassay (RIA) [2]. Nonspecific binding was defined as bound radioactivity in the presence of 50–100 M excess of unlabeled ligand [2].

Growth Assays

After time periods ranging from 1 to 12 days, cells grown in culture in the presence or absence of growth factors were trypsinized and cell numbers were determined using a Coulter cell counter [3].

RESULTS AND DISCUSSION

Table I identifies MAbs to the growth factor receptors for epidermal growth factor (EGF), insulin-like growth factor (IGF)-I, and nerve growth factor (NGF), all of which inhibit binding of the corresponding growth factor to the respective target cells. Six melanoma cell lines grown in a culture medium devoid of polypeptide growth factors (protein-free medium) were used in our studies. As shown in Figure 1 for melanoma cell line WM 239-A, insulin, EGF, and a combination of insulin, transferrin, and EGF significantly stimulated growth when compared to cultures deprived of growth factors. Growth stimulation by insulin could be demonstrated in cultures of all of five metastatic melanoma cell lines and by EGF in cultures of three out of five cell lines [3].

Epidermal Growth Factor Receptor

EGF-induced stimulation of growth of cells of four out of six melanoma cell lines occurred regardless of the expression of EGF receptors on the cell membrane of these cells (Table II). The lack of receptor expression may be explained by internalization of the receptor after ligand binding. There is no evidence that EGF is produced by melanoma cells in culture (unpublished data). However, transforming growth factor alpha (TGFα) is secreted by various melanoma cells [7–10]. TGFα competes with EGF for binding to the EGF receptor [7]. The hitherto undetermined amount of TGFα produced by our cells may contribute to the continuous downregulation of the EGF receptor on those cells.

TABLE I. Growth Factor Receptors Detected by Monoclonal Antibodies Used in Our Studies

Growth factor receptor	MAb	Isotype	Reference	Ligand binding inhibition	Growth modulation
IGF-I receptor	αIR-3	IgG1	[4]	Yes	ND[a]
EGF receptor	425	IgG2a	[2,5]	Yes	Yes
NGF receptor	ME 20.4	IgG1	[6]	Yes	No
	ME 82-11	IgG1	[6]	Yes	No

[a]ND = not determined.

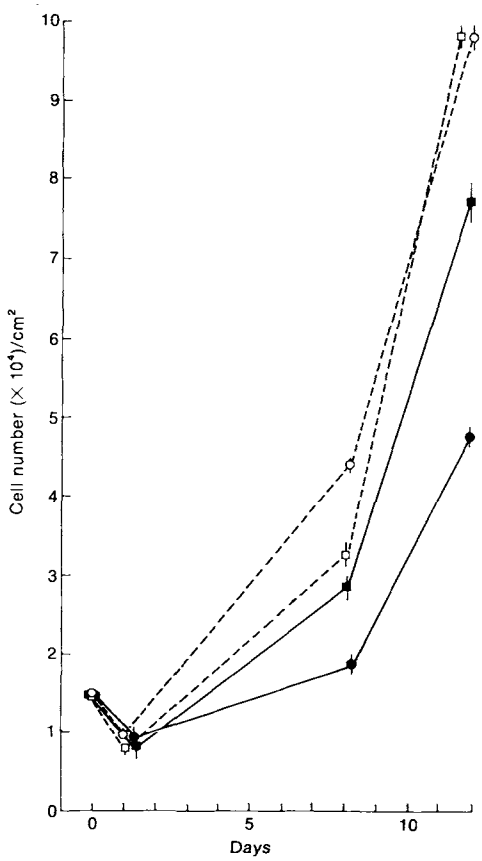

Fig. 1. Growth stimulation of metastatic melanoma cell line WM 239-A by EGF (1 nM) (■---■), insulin (1 μM) (□---□), and a combination of EGF, insulin, and transferrin (125 nM) (○---○). Cell numbers of cultures grown for 8 and 12 days in the presence of each growth factor were significantly ($p < 0.05$ in Student's t-test) higher than cell numbers of cultures grown in protein-free base medium (●---●). Results are given as mean cell counts ± SD of duplicate cultures.

The effect of anti-EGF receptor MAb 425 on EGF-receptor-expressing target cells was studied with human epidermoid carcinoma cell line A 431, which has $> 10^6$ EGF binding sites per cell [2]. Similar to growth inhibition induced by EGF, A 431 cells were growth inhibited by MAb 425 present in the culture medium at concentrations greater than 1 nM. As shown in Figure 2, growth inhibition of A 431 cells by MAb 425 was accompanied by a decrease in the number of cells in S-phase of the cell cycle, suggesting that the antibody induces specific alterations of the cell cycle.

Insulin and Insulin-Like Growth Factor Receptors

In order to study growth effects of insulin and IGF-I on melanoma cells, we used MAb αIR-3 (see Table I), which reacts with the human type I IGF receptor and blocks binding of insulin and IGF-I to the receptor. MAb αIR-3 partially inhibited growth stimulation of cells of three melanoma cell lines by both insulin and IGF-I (Fig. 3). These results strongly suggest that growth stimulation of melanoma cells induced by insulin is mediated via the type I IGF receptor.

TABLE II. Lack of Correlation Between EGF-Receptor Expression and Growth Stimulation of Melanoma Cells Induced by EGF*

Cell line	EGF-R expression[a]	Growth stimulation by EGF[b]
WM 266-4[c]	0	Yes
WM 239-A[c]	0	Yes
WM 373	0	No
WM 164	0	Yes
WM 115[c]	15	No
WM 852	45	Yes

*Melanoma cells used in these experiments have been adapted to grow in serum-free (WM 115) or protein-free medium.
[a]Expression assessed in mixed hemadsorption assays with MAb 425; results expressed as % rosette-forming cells. Similar results were obtained in radioimmunoassays using ^{125}I-labeled EGF and MAb 425.
[b]Stimulation of growth was determined in cell counting assays; wells containing EGF (5 ng/ml) were scored positive if they contained significantly ($p < 0.05$ in Student's t-test) higher No. of cells than did wells with medium devoid of exogenously added growth factors.
[c]Primary (WM 115) and metastatic (WM 239-A, WM 266-4) melanoma cell lines derived from the same patient.

Nerve Growth Factor Receptor

Although normal melanocytes and cells of metastatic melanoma lines in culture express the NGF receptor [11], none of the six cultured melanoma cells studied were growth stimulated by NGF (not shown). This is not surprising since NGF-induced effects of target tissues of neuroectodermal origin are rather related to survival and differentiation [12,13]. Its effect on melanoma cells may thus be related to the pathways of differentiation in situ and in vitro [11].

Other Growth Factor Receptors

Various other growth factors seem to be relevant in the growth regulation of melanocytic cells. Halaban et al [14] have shown that basic fibroblast growth factor (FGF) has mitogenic effects on normal melanocytes. Platelet-derived growth factor (PDGF) [15], TGFα [7–10], and TGFβ [8] are the better characterized growth-modulating activities produced by melanoma cells, but their effects on growth of the cells still await detailed investigation. Equally, the corresponding receptor for melanoma growth-stimulating activity described by Richmond et al [10] remains to be identified.

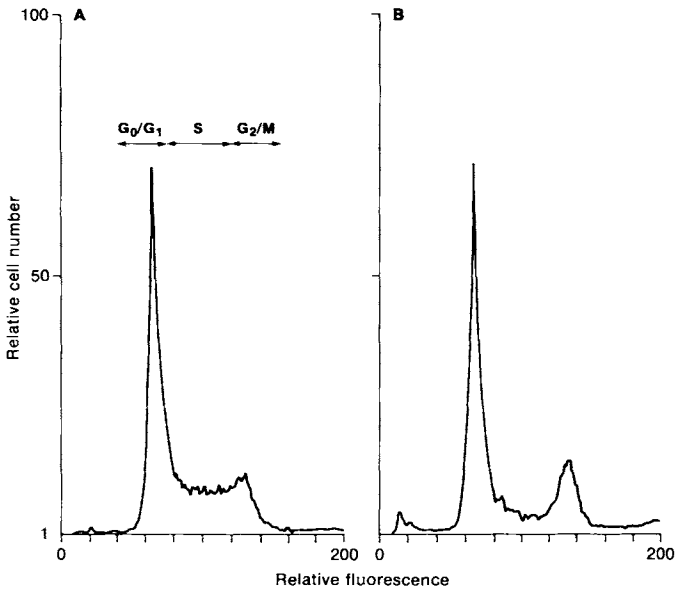

Fig. 2. Changes in cell cycle distribution of A 431 epidermoid carcinoma cells incubated with anti-EGF receptor MAb 425. **A** shows the cell cycle distribution of cells in untreated cultures; **B** demonstrates a clear decrease of the number of cells in S-phase in cultures treated with 32 nM MAb 425 after 4 days of culture. Decrease in S-phase cells in antibody treated cultures was statistically significant ($p < 0.05$) in Student's t-test when compared to control cultures.

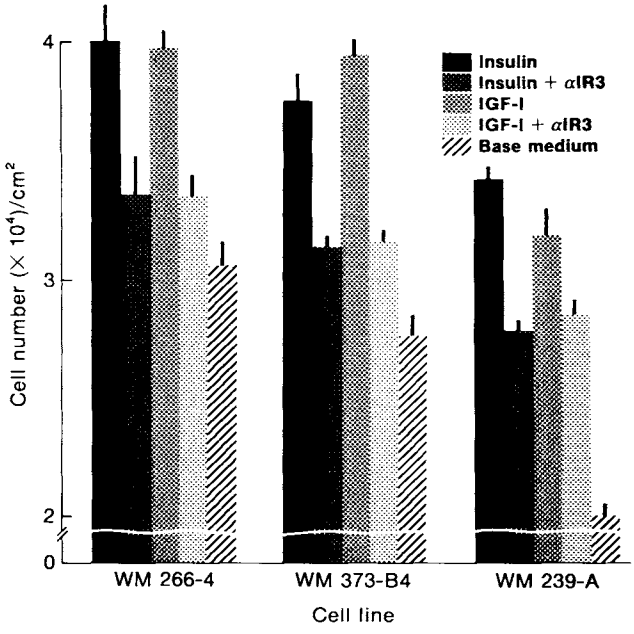

Fig. 3. Inhibition of insulin (1 μM)- and IGF-I (13 nM)-mediated growth stimulation in three malignant melanoma cell lines by MAb αIR3 (100 nM), which reacts with the type I IGF receptor. Results are given as mean cell counts ± SD of duplicate cultures.

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REFERENCES

1. Herlyn M, Guerry D, Koprowski H: J Immunol 134:4226, 1985.
2. Rodeck U, Herlyn M, Herlyn D, Molthoff C, Atkinson B, Varello M, Steplewski Z, Koprowski H: Cancer Res 47:3692, 1987.
3. Rodeck U, Herlyn M, Menssen HD, Furlanetto RW, Koprowski H: Int J Cancer (in press).
4. Jacobs S, Cook S, Svoboda ME, Van Wyk JJ: Endocrinology 118:223, 1986.
5. Murthy U, Basu A, Rodeck U, Herlyn M, Ross A, Das M: Arch Biochem Biophys 252:549, 1987.
6. Ross AH, Grob P, Bothwell M, Elder D, Ernst CS, Marano N, Ghrist BFD, Slemp CC, Herlyn M, Atkinson B, Koprowski H: Proc Natl Acad Sci USA 81:6681, 1984.
7. Marquard H, Todaro GJ: J Biol Chem 257:5220, 1982.
8. DeLarco JE, Pigott DA, Lazarus JA: Proc Natl Acad Sci USA 82:5015, 1985.
9. Kim MK, Warren TC, Kimball ES: J Biol Chem 258:6561, 1985.
10. Richmond A, Lawson DH, Nixon DW, Chawla, RK: Cancer Res 45:6390, 1985.
11. Herlyn M, Clark WH, Rodeck U, Mancianti ML, Jambrosic J, Koprowski H: Lab Invest 56:461, 1987.
12. Edgar D: J Cell Sci [Suppl] 3:107, 1985.
13. Dichter MA, Tischler AS, Greene LA: Nature 268:501, 1977.
14. Halaban R, Ghosh S, Baird A: In Vitro Cell Dev Biol 23:47, 1987.
15. Westermark B, Johnsson A, Paulsson Y, Betsholtz C, Heldin C-H, Herlyn M, Rodeck U, Koprowski H: Proc Natl Acad Sci USA 83:7197–7200, 1986.