Growth Factor Binding to 7,12-Dimethylbenz(a)anthracene-induced Mammary Tumors from Rats Subject to Chronic Caloric Restriction

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ABSTRACT

Caloric restriction (CR) inhibits tumorigenesis in rodents. To understand the basis for this effect the binding of insulin, insulin-like growth factor I (somatomedin C [IGF-I/Sm-C]), insulin-like growth factor II (multiplication stimulating activity [IGF-II/MSA]), and epidermal growth factor were examined to membrane preparations of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary adenocarcinomas and several normal tissues from female Sprague-Dawley rats. Animals were fed ad libitum (AL) or 25% and 40% calorically restricted diets. Large, palpable (LP) and small, ≤ 100 mg, non-palpable (SNP) tumors were evaluated. Growth factor binding to tumors was differentially affected by CR. IGF-I/Sm-C binding was comparable for AL-LP, AL-SNP, and 25% CR-LP tumors, but elevated in 25% CR-SNP tumors. Scatchard analysis revealed high and low affinity IGF-I/Sm-C binding sites, with AL-SNP and 25% CR-SNP tumors exhibiting similar levels of high affinity sites and at a greater concentration than AL-LP and 25% CR-LP tumors. Insulin binding to mammary tumors was low, i.e., 8- to 13-fold lower than IGF-I/Sm-C binding. The 25% CR-LP and SNP tumors bound 2- to 5-fold more insulin than corresponding AL-LP and SNP tumors. Binding of IGF-II/MSA to these tumor preparations was high, approximately 11- to 25-fold greater than insulin binding, and was unaffected by CR or tumor size. The binding of epidermal growth factor was not detected in any tumor preparations. Receptor binding studies were confirmed with covalent cross-linking and sodium dodecyl sulfate-polyacrylamide gel electrophoresis analyses. Normal tissues exhibited tissue- and growth factor-specific alterations in binding with host CR. Thus, alterations in growth factor binding were not tumor specific, but were less pronounced than in mammary tumors. These findings suggest alterations in IGF-I/Sm-C and insulin binding properties to tumors in relation to CR and tumor size may contribute, in part, to the inhibitory effects of CR on tumorigenesis.

INTRODUCTION

The peptide GF insulin, IGF-I/Sm-C, IGF-II/MSA, and EGF have been shown to exert potent metabolic and mitogenic effects on a variety of normal and neoplastic cell types in vitro and in vivo. The precise mechanism(s) of action of these growth factors and their mitogenic signal transduction networks are incompletely understood, but a crucial first step involves GF binding to specific, saturable cell-surface receptors. IGF-I/Sm-C binds preferentially to the type I IGF receptor, with IGF-II/MSA, insulin, and insulin binding properties of host CR. The experimental design and dietary regimen used in tumorigenesis studies are as described in the preceding report (9). The experimental protocol was approved by the Wistar Institute's Institutional Animal Care and Use Committee. At termination of the study, mammary tumors and normal tissues were obtained as described in the previous paper (9).

MATERIALS AND METHODS

Hormones, growth factors, and other biologicals used were prepared or obtained as described in the previous paper (9). The peptide GF insulin, IGF-I/Sm-C, IGF-II/MSA, and EGF were examined for expression of these factors as described in the preceding report (9). The experimental protocol was approved by the Wistar Institute's Institutional Animal Care and Use Committee. At termination of the study, mammary tumors were obtained as described in the previous paper (9).

Membrane Preparation

Plasma membrane-enriched fractions of tumors were prepared by differential centrifugation according to the method of Cuarescas (22). After weighing, tissues were pooled to approximately 1 g wet weight. Briefly, tissues were minced, homogenized in several volumes of cold buffer (0.25 M sucrose, 5 mM EDTA, 0.1 mM PMSF and 10 KIU/ml aprotinin) with a Polytron (Brinkman Instruments, Westbury, NY) at setting 7 for 4 x 10-s bursts at 4°C. Homogenates were subjected to two low speed centrifugations (375 x g) at 4°C and the pooled supernatants centrifuged at 6000 × g for 60 min at 4°C. Supernatants were incubated with 0.1 M NaCl and 1.0 mM MgsO₄ at room temperature for 15 min, and centrifuged at 40,000 × g for 40

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4The abbreviations used are: CR, calorie restriction; IGF-I/Sm-C, insulin-like growth factor I/somatomedin C; IGF-II/MSA, insulin-like growth factor II/multiplication-stimulating activity; EGF, epidermal growth factor; AL, ad libitum; LP, large, palpable tumors; SNP, small, nonpalpable tumors ≤ 100 mg; GF, growth factor; BSA, bovine serum albumin; PMSF, phenylmethylsulfonyl fluoride; hIGF₁, human insulin-like growth factor I; SDS-PAGE sodium dodecyl sulfate-polyacrylamide gel electrophoresis; DMBA, 7,12-dimethylbenz(a)-anthracene.
min at 4°C. The high speed pellets were washed once with resuspension buffer (50 mM Tris-HCl, 5 mM EDTA, 0.1 mM PMSF, 10 KIU/ml aprotinin) and after centrifugation resuspended in this buffer using a glass pestle. Membrane aliquots were stored at —70°C. Membrane protein concentrations were determined by a modified Lowry method (23). Plasma membrane-enriched fractions of beef liver and human placenta were similarly prepared as controls for binding studies.

Hormone and Growth Factor Binding Studies

IGF-I/Sm-C Binding Assay. The binding of 125I-IGF-I (specific activity, ~250-300 µCi/µg) was measured by adding ~30,000 cpm (~0.025 nm) of 125I-IGF-I/Sm-C to duplicate aliquots of membrane fractions (25-275 µg protein) in IGF binding buffer consisting of 50 mM Tris-HCl, 0.25% BSA, 0.02% sodium azide, pH 8.0, in a total assay volume of 0.5 ml. Nonspecific binding was assessed by incubating samples with a 250- to 500-fold molar excess of pure recombinant hIGF-G, or 100 nM of an IGF preparation of 4% purity containing three parts IGF to two parts IGF-II (3). The contribution of IGF-I/Sm-C binding to IGF binding protein was assessed using a 500- to molar excess of porcine insulin. Human placental membranes and bovine liver membranes were used as high and low IGF-I/Sm-C binding controls, respectively. Variability of binding data for all growth factors assessed was <10%. Samples were incubated overnight at 4°C and receptor-bound and free radioligand separated using the polyethylene glycol-1% human immunoglobulin G precipitation technique previously described (24). Membrane pellets were counted as previously detailed (9). Specific binding was measured as the difference between total binding (in absence of unlabeled ligand) and nonspecific binding, and is expressed as percentage of total counts added. All binding assays, including those described below, were repeated a minimum of three times.

IGF-II/MSA Binding Assay. The binding of 125I-IGF-II/MSA (specific activity, ~150-300 µCi/µg) was measured by the addition of ~30,000 cpm (~0.027 nm) of 125I-IGF-II to duplicate membrane fractions over a range of protein concentrations in IGF binding buffer as described above for IGF-I/Sm-C binding. Total assay volume was 0.5 ml. Nonspecific binding was assessed using 100 nM of the impure IGF fraction described above, or a 250- to 500-fold molar excess of pure IGF-II. Incubation conditions, separation of receptor-bound and free radioligand, and quantitation were as described for IGF-I/Sm-C binding assays.

Insulin Binding Assay. The binding of 125I-insulin (specific activity, 2200 Ci/mmol) to duplicate membrane fractions was measured by the addition of ~50,000 cpm (~0.027 nm) of 125I-insulin in a modification of the buffer system of Feldman and Hilf (25). The binding buffer consisted of 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1% BSA, 1.0 mM CaCl2, 1 mg/ml bacitracin, and 0.02% sodium azide, pH 7.4. Nonspecific binding was assessed with a 500-fold molar excess of unlabeled hIGF-I/Sm-C. In addition to 125I-IGF-I/Sm-C binding, an excess (1:500 dilution) of anti-IGF-I/Sm-C antibody was utilized (3). Incubation conditions and separation of receptor-bound and free ligand were as above.

Insulin binding by tumor plasma membranes was done in insulin binding buffer with ~60,000 cpm (~0.034 nm) 125I-insulin over a range of 2.0 x 10^-12 M to 1.0 x 10^-8 M unlabelled porcine insulin (see Ref. 25). Nonspecific binding was assessed using a 1000-fold molar excess of unlabelled insulin.

Saturation binding data was transformed for Scatchard analysis as detailed by Boeynaems and Dumont (27). Data were analyzed using the mathematical modeling program, PROPHET (Bolt Beranek and Newman Inc., Cambridge, MA), to obtain estimates of the concentration and affinity of binding sites.

Chemical Cross-Linking of Growth Factors to Membrane Preparations. Radiolabeled growth factors (1.0-2.5 x 10^8 cpm/tube) were chemically cross-linked to plasma membrane preparations using 0.2 mM disuccinimidyl suberate dissolved in dimethyl sulfoxide as described (2, 3). Reactions were carried out at 4°C and terminated after 15 min with 1 mM Tris-HCl, pH 8.8. Cross-linked preparations were pelleted and solubilized with sample buffer containing 0.3% SDS and analyzed under reduced (100 mM dithiothreitol) and nonreduced conditions by SDS-PAGE using the discontinuous buffer system of Laemmli (28). The dried gels were examined autoradiographically using Kodak X-Omat AR-5 film after incubation at —70°C for ~3-10 days.

RESULTS

In this study, GF binding properties have been examined in LP and SNP DMBM-induced mammary tumors (9) from rats subject to AL feeding and 25% CR regimens. We have examined whether host CR altered tumor GF binding in more rapidly developing LP tumors and the presumably growth-inhibited SNP tumors within each treatment group. Despite several CR studies, insufficient nonnecrotic tumor tissue was available from 40% CR rats for biochemical analyses (see Ref. 29).

The binding of 125I-IGF-I/Sm-C to DMBM-induced mammary tumors over a range of protein concentrations is shown in Fig. 1. IGF-I/Sm-C binding was comparable for LP and SNP tumors from AL-fed rats and LP tumors from 25% CR rats over a range of membrane protein concentrations, but was elevated in the SNP tumors from restricted animals. The percentage of specific binding of IGF-I/Sm-C to human placental membranes (high control) was 24.6 ± 1.3% (SD) and to bovine

Fig. 1. Specific binding of 125I-IGF-I/Sm-C to plasma membrane-enriched preparations of DMBM-induced mammary tumors. SNP tumors from 25% CR rat (A); SNP tumors from AL-fed rats (O); LP tumors from 25% CR rats (O); LP tumors from AL-fed rats (*). Binding experiments were performed as described in "Materials and Methods." Values shown are means of duplicate samples from three experiments.

4136
Scatchard transformation of IGF-I/Sm-C equilibrium data to the tumors is shown in Fig. 2, A and B. Scatchard plots were curvilinear suggesting the existence of both high and low affinity IGF-I/Sm-C binding sites on these mammary tumors. Estimations of the concentrations and equilibrium binding constants (Kd values) of these sites are described in the legend of Fig. 2.

It appears that SNP tumors from AL-fed and 25% CR animals have a similar number of high affinity sites for IGF-I/Sm-C on a fmol/μg membrane protein basis and at a greater concentration than the corresponding LP tumors from AL-fed and 25% CR rats. Of interest was the observation that the predominant IGF-Sm-C binding sites on the SNP tumors from 25% CR rats (Fig. 2) were high affinity sites. While the presence of some low affinity sites is likely, they could not be detected in three independent experiments. It appears that there are alterations in IGF-I/Sm-C binding properties to tumors as a function of both host CR and tumor size.

Covalent cross-linking studies with radiolabeled IGF-I/Sm-C to tumor membranes and normal tissues under reducing conditions (Fig. 3, A and B) revealed a binding moiety of ~130,000 molecular weight characteristic of the ligand-binding α-subunit of the type I receptor. This binding was inhibited completely by excess unlabeled IGF-I/Sm-C, but incompletely by equimolar insulin concentrations, particularly for LP tumors from 25% CR animals. The dense bands in the upper portion of the autoradiographs are cross-linked dimers of the type I receptor’s α-subunits (3). Moreover, several diffuse lower mo-

![Fig. 2. Scatchard transformation of $^{125}$I-IGF-I/Sm-C binding data to plasma membrane-enriched preparations of DMBA-induced mammary tumors. A, SNP tumors from 25% CR rats (○). $B_{max} = 346$ fmol/μg protein; $K_d = 0.184$ nM. SNP tumors from AL-fed rats (□). $B_{max} = 345$ fmol/μg protein; $K_d = 0.028$ nM; $B_{max} = 2.65$ pmol/μg protein; $K_d = 4.25$ nM. B, LP tumors from 25% CR rats (○). $B_{max} = 1.11$ pmol/μg protein; $K_d = 0.011$ nM; $B_{max} = 1.11$ pmol/μg protein; $K_d = 1.17$ nM. LP tumors from AL-fed rats (☆). $B_{max} = 197$ fmol/μg protein; $K_d = 0.049$ nM. $B_{max} = 1.4$ pmol/μg protein; $K_d = 2.8$ nM. Binding experiments and Scatchard transformation of equilibrium binding data were as described in “Materials and Methods.” Values shown are means of duplicate samples from three experiments.](image)

![Fig. 3. Autoradiogram of $^{125}$I-IGF-I/Sm-C-labeled binding species present in plasma membrane-enriched preparations of DMBA-induced mammary tumors (A) and representative normal tissues (B) from AL, 25% CR, and 40% CR rats. Cross-linking procedures are described in “Materials and Methods.” Samples were reduced with 100 mM dithiothreitol before electrophoresis on a 5-15% gradient polyacrylamide gel. Numbers at left, position and sizes (in $M_x 10^3$) of molecular weight marker proteins. Exposure time was 5 days at $-70°C$. A, membrane preparations of AL-LP tumors incubated with 2.4 nM $^{125}$I-IGF-I/Sm-C in the absence (lanes a, c) or presence of 240 nM unlabelled IGF-I/Sm-C (lane b) or 240 nM unlabelled insulin (lane d). Lanes e and g contain 25% CR LP tumor preparations incubated with 2.4 nM $^{125}$I-IGF-I/Sm-C alone and in the presence of 240 nM unlabelled IGF-I/Sm-C (lane f) or 240 nM unlabelled insulin (lane h). B, normal tissues with 2.4 nM $^{125}$I-IGF-I/Sm-C alone; Lane a, 25% CR liver; lane d, 240 nM unlabelled IGF-I/Sm-C in the presence of 240 nM unlabelled IGF-I/Sm-C (lane j) or 240 nM unlabelled insulin. Lane a, 25% CR liver; lane d, 240 nM unlabelled IGF-I/Sm-C alone; Lane e, 25% CR liver; lane e, 240 nM unlabelled insulin (lane d). Lane g, 40% CR kidney; lane j, AL heart; lane j, AL lung. Tissue preparations incubated with 2.4 nM $^{125}$I-IGF-I/Sm-C in the presence of 240 nM unlabelled IGF-I/Sm-C; lane b, 25% CR liver; lane e, 240 nM unlabelled IGF-I/Sm-C alone; Lane a, 25% CR liver; lane d, 240 nM unlabelled insulin (lane d). Lane g, 40% CR kidney; lane j, AL heart; lane j, AL lung.](image)
lecular cross-linked to high affinity, low molecular weight IGF binding protein moieties (2, 3). A species of ~40,000 molecular weight appears quite intense in LP tumors from AL-fed rats, but is absent in LP tumors from 25% CR animals. The remaining binding species appear comparable for LP tumors from AL-fed and 25% CR animals. Lack of availability of membrane preparations from SNP tumors precluded cross-linking studies. Competitive inhibition of IGF-I/Sm-C binding to the type I receptor could not be examined in greater detail.

Insulin binding to these mammary tumors (Fig. 4) was assessed in a number of experiments and was very low, i.e., 8- to 13-fold lower than IGF-I/Sm-C binding at comparable membrane protein concentrations. The physiological relevance of such low insulin binding to this tumor remains to be clarified (see “Discussion”). Despite overall low binding, LP and SNP tumors from 25% CR rats bound 2- to 5-fold more insulin than the corresponding LP and SNP tumors from AL-fed rats. Human placental membranes bound 20.2 ± 2.5% (SD) radiolabeled insulin under the same binding conditions, while bovine liver membranes exhibited 2.4 ± 0.4% (SD) insulin binding.

Scatchard transformation of subsequent saturation binding data was attempted, but due to low insulin binding, accurate estimations of the number and affinity of insulin binding sites were not possible (data not shown). Covalent cross-linking studies with radiolabeled insulin to LP tumors and controls revealed a binding moiety of ~130,000 molecular weight characteristic of the reduced insulin-binding α-subunit of the insulin receptor, although resolution was poor (data not shown). Binding was completely inhibited by excess unlabeled insulin. High molecular weight α-subunit dimers were likewise identified in the autoradiographs, but no binding to lower molecular weight moieties was observed.

The percentage radiolabeled IGF-II/MSA specifically bound to these mammary tumors (Fig. 5) was high, i.e., up to twofold greater than IGF-I/Sm-C binding and 11- to 25-fold greater than insulin binding over comparable membrane protein concentrations. No significant differences were observed as a function of CR. Specific binding to beef liver membranes was 46.0 ± 2.5% (SD) in these studies. Lack of availability of sufficient quantities of pure IGF-II/MSA at the time of these experiments, limited tumor material, precluded competitive binding studies and Scatchard analysis.

Covalent cross-linking studies with radiolabeled IGF-II/MSA (Fig. 6, A and B) under reduced and nonreduced conditions revealed that this binding was likely to the monomeric type II receptor based on its altered electrophoretic mobility on gradient polyacrylamide gels (see Refs. 3 and 4). Under nonreducing conditions a dense band of ~220,000 molecular weight was visible, while after reduction, a higher molecular weight moiety was observed (Fig. 6B). Curiously, no detectable binding of radiolabeled IGF-II/MSA to the type I receptor or lower molecular weight IGF binding species was observed. The binding of radiolabeled IGF-II/MSA was inhibited by excess unlabeled pure IGF-II/MSA or an impure IGF mixture. These collective findings suggest that the IGF-II/MSA binding observed was largely, if not exclusively, to the type II receptor on these tumors.

The binding and cross-linking of radiolabeled EGF to these mammary tumors was virtually nondetectable (<1.0% specifically bound, data not shown). Despite alterations in plasma membrane preparation, variations in binding protocols and buffer conditions, and use of a range of radiolabeled EGF concentrations, the highest specific binding obtainable was ≤0.0%. A treatment (26) to remove possible endogenously produced EGF-like factors was employed with no improvement in binding. These findings suggest little, if any, EGF responsiveness of these tumors and await further clarification.

The binding and cross-linking of radiolabeled GFs to a variety of normal tissues was evaluated to ascertain whether alterations in binding properties were tumor specific or a generalized consequence of host CR. As depicted in Table 1, alterations in binding properties as a function of CR were varied and tissue specific. Quantities of pure IGF-II/MSA at the time of these experiments and limited tumor material, precluded competitive binding studies and Scatchard analysis.

Fig. 5. Specific binding of 125I-IGF-II/MSA to plasma membrane-enriched preparations of DMBA-induced mammary tumors. SNP tumors from AL-fed rats (▵); SNP tumors from AL-fed rats (○); LP tumors from 25% CR rats (□); LP tumors from AL-fed rats (*). No SNP tumors from 25% CR rats were available. Binding experiments were performed as described in “Materials and Methods.” Values shown are means of duplicate samples from three experiments.

Fig. 4. Specific binding of 125I-insulin to plasma membrane-enriched preparations of DMBA-induced mammary tumors. SNP tumors from AL-fed rats (▵); SNP tumors from AL-fed rats (○); LP tumors from 25% CR rats (□); LP tumors from AL-fed rats (*). Binding experiments were performed as described in “Materials and Methods.” Values shown are means of duplicate samples from three experiments.

DISCUSSION

In this study we have reported the binding properties of insulin-like growth factors and EGF to DMBA-induced rat
mammary adenocarcinomas. Likewise, we have reexamined the insulin binding properties of this neoplasm. Finally, this is the first report of alterations in GF binding to tumor tissue and normal tissues as a function of restricted caloric intake.

IGF-I/Sm-C binding to mammary tumors was altered, both number of sites and affinities, as a function of host CR and tumor size. In contrast, fasting serum IGF-I/Sm-C levels in vivo were normalized throughout the latter 75% of the 5-month CR studies (9). These data suggest that tumor tissue has an impaired ability to properly regulate ligand-binding capacity, thereby resulting in an altered sensitivity to circulating growth factor or hormone (see Ref. 30).

The IGFs have been implicated in mammary carcinoma growth. Furlanetto and DiCarlo (2) demonstrated that several human breast cancer cell lines (MCF-7, T47-D, MDA-MB-231, and HLB-100) possess type I receptors with $K_a$ values ranging from 0.5 to 4 nM. Physiological levels of IGF-I/Sm-C stimulated DNA synthesis through the type I receptor in all four cell lines although to varying degrees, as additional serum factors were required for optimal growth. Huff et al. (31) extended these findings by demonstrating that IGF-I/Sm-C-responsive breast cancer cell lines secreted the GF into their medium, with estrogen-independent lines secreting significantly more than estrogen-dependent cells. The presence of type I and type II IGF receptors has been demonstrated in vitro on human breast cancer cell lines (32). Pekonen et al. (33) observed that IGF-I/Sm-C binding to human breast tumors was increased significantly compared to normal breast tissues, suggesting a relationship between expression of the type I receptor and malignant transformation of mammary epithelium. Moreover, IGF-I/Sm-C binding to tumor tissue was significantly correlated with estrogen- and progesterone-receptor status, and app-
GROWTH FACTOR BINDING AND CALORIE RESTRICTION

Table 1 Growth factor binding to plasma membrane-enriched fractions of normal tissues from rats fed ad libitum 25% and 40% calorically restricted diets

Results are expressed based upon percentage of radiolabeled growth factor specifically bound. Membrane protein concentration (μg) for tissues: liver, 108-121; kidney, 121; heart, 113-120; lung, 105-125; skeletal muscle, 75. Details of binding studies are described in "Materials and Methods." Values are based on results of three separate experiments.

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<td>Insulin</td>
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* Asterisks denote ranges for percentage-specific binding: *, (10 < 15); **, (15 < 20); ***, (20 < 25); ****, (25 < 30); ***** (30 < 35).
* NA not available for analysis.

peared to be a more general property of breast tumors than EGF receptor expression.

The low level of insulin binding to these mammary tumors was surprising in view of several reports on the growth-stimulating properties of insulin on DMBA-induced mammary tumors. Heuson et al. (18) demonstrated that approximately 90% of DMBA-induced mammary tumors were insulin dependent. Alloxan-induced diabetes in rats treated with DMBA caused a rapid regression of tumor growth similar to that following oophorectomy or hypophysectomy; inhibition of tumor growth was reversed by insulin administration (18). The studies of Hilf and coworkers (19, 20) revealed that a majority of DMBA-induced mammary tumors were hormone-dependent, regressing in diabetic or ovariecetomed rats with tumors from diabetic animals showing increased insulin binding, but reduced estrogen receptor status.

Our findings for insulin and IGF-I/Sm-C binding to mammary tumors are not necessarily incompatible with those reported above. At the time of the earlier studies (18–21), the IGFs and their specific receptors were not well characterized. Insulin can function as a growth factor directly through its own receptor, or through cross-reacting with the structurally related type I receptor in a number of cell types (see Ref. 2). In view of our earlier findings (9) and the results reported here, we suggest that the in vitro and in vivo growth responsiveness of DMBA-induced rat mammary carcinomas to insulin may be due, in part, to indirect effects of insulin on IGF-I/Sm-C levels, or due to the interaction of insulin with the more abundant type I receptors on these tumors.

The binding of IGF-II/MSA to these mammary tumors was high, surpassing that of the high control employed in these studies. Type II receptors have been characterized in a number of human breast cancer cell lines (32) but not in carcinoegenduced mammary tumors. Significant alterations in IGF-II/MSA binding to tumors as a function of CR and tumor size were not observed, nor did circulating levels of IGF-II/MSA show a significant pattern of alteration in response to chronic CR (9). Elevations in tumor IGF-II/MSA binding are not surprising in view of the less differentiated nature of neoplastic tissues.

The binding of EGF to DMBA-induced mammary carcinomas was reported by Zweibel et al. (34) who found high affinity EGF binding in primary cultures although receptor levels were one-third that of normal mammary epithelial cells. Ether and Cundiff (17) reported the GF requirements of epithelial cells derived from DMBA-induced or NMu-induced rat mammary carcinomas in vitro. Of the tumors examined, 50% demonstrated GF independence, and of these greater than 50% were not dependent on insulin, EGF, or cholera toxin for growth.

EGF receptor expression seems to vary between subgroups of human breast carcinomas (33, 35), and a significant percentage of human and rodent mammary tumors may be EGF independent. These observations contrast with the stimulatory role demonstrated for EGF on mammary tumors in mice (36, 37). Further detailed characterization of EGF receptor expression and regulation in the carcinogen-induced rat mammary tumor model described here are in progress.

In conclusion, we have demonstrated that GFs bound to DMBA-induced rat mammary carcinomas to varying degrees, with IGF-II/MSA binding to a similar or slightly greater extent than IGF-I/Sm-C, and both IGFs binding to a far greater extent than insulin. EGF binding to this neoplasm was not detectable. CR and tumor size each were independently associated with alterations in the number and affinity of receptors for IGF-I/Sm-C and insulin, but not with changes in the type II receptor for IGF-II/MSA. These alterations with chronic CR of the host were not tumor specific, but affected a variety of normal tissues as well. The findings reported here and previously (9) suggest the possibility that alterations in certain peptide GFs, their receptor status on target tissues, and complex interactions between these, may contribute, in part, to the inhibition of tumor growth by CR. The potential role of autocrine or paracrine influences of the GFs examined on tumor growth in CR animals is being investigated. These influences, and alterations in circulating mammotrophic hormones and/or their receptors as a function of host CR may explain the pronounced inhibitory effects of this nutritional intervention on mammary tumorigenesis.

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