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Insulin receptor tyrosine kinase activity in colon carcinoma

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Abstract

Colon carcinoma is the most common tumor of the gastrointestinal tract. According to some investigators, insulin, epidermal growth factor (EGF) and insulin-like growth factor I (IGF-I) may be involved in the neoplastic proliferation. Insulin-binding and receptor tyrosine kinase activity were investigated in colon carcinomas and in normal colons. The insulin receptor concentration, as shown by binding assays, was 17.4 ± 4.3 fmol/µg in normal colon and 29.69 ± 9.4 fmol/µg in colon carcinoma. Nevertheless, the insulin affinity of the receptor was similar in both groups (Kd ≅ 1 nM). Both normal and neoplastic colon showed phosphorylation of the insulin receptor. The electrophoretic migration of the ß-subunit of the insulin receptors purified from colon carcinomas was similar to that of normal colon and both tissues demonstrated an insulin-dependent autophosphorylation. The receptor tyrosine kinase activity was measured by the incorporation of [gamma^{32}P]ATP into the ß-subunit. The basal and the insulin-stimulated tyrosine kinase activities were significantly higher in colon carcinomas compared to normal colon tissues (2.2 and 1.6 times, respectively). Understanding the metabolism of neoplastic cells may contribute to the development of prevention strategies as well as new therapies. It is now necessary to study other steps of the insulin signal transduction pathway, such as insulin receptor substrate 1 phosphorylation.

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- Colon carcinoma
- Insulin receptor
- Tyrosine kinase
- Insulin signaling

Introduction

Insulin has a broad spectrum of effects on target cells. Besides its well-known metabolic actions, insulin exerts growth-promoting effects such as stimulation of RNA and DNA synthesis (1,2). Although there is evidence that insulin plays an important role in the proliferation of colon tumor cells *in vitro* (3,4), it is not known which molecular mechanisms are involved and how the insulin receptor participates in this process. The insulin receptor consists of two α- and two ß-subunits that are bound by disulfide bridges. The α-subunits (MW 135 kDa) located outside the plasma membrane are the binding sites for insulin. The ß-subunits are 95-kDa transmembrane proteins with intrinsic tyrosine kinase activity. The binding of insulin to the α-subunit of the receptor activates the kinase activity of the ß-subunit (5). It is believed that further signal transduction occurs through phosphorylation of cellular proteins at tyrosine residues, which could transmit the insulin signal to the metabolic effector systems of the target cell (6).

In the present study the insulin receptors of 5 human colon carcinomas and 5 adjacent

normal colon tissues were isolated by chromatography on a wheat-germ-agglutinin (WGA) column. Specific binding assays of [^{125}I]-insulin to WGA-purified insulin receptors and tyrosine kinase activity assays were performed.

Material and Methods

Tissue samples

Samples of colon carcinoma were obtained at the time of surgery, were immediately frozen in liquid nitrogen and stored at -80°C until preparation. We studied the tumor and adjacent normal colon from 5 patients. Carcinoma and normal regions of colon tissue were identified histologically.

Receptor preparation

Normal and tumor tissues were homogenized with a cell disruptor for 10 to 15 sec with maximal speed at 4°C in the presence of the protease inhibitors phenylmethylsulfonylfluoride (2.5 mM) (Sigma), aprotinin (1200 trypsin inhibiting units/l) (Sigma), benzamidine (10 mM) (Sigma), bacitracin (7500 U/l) (Sigma) in 25 mM HEPES (Sigma) buffer, pH 7.4. Subsequently the lysate was centrifuged for 50 min at 200,000 *g* at 4°C. The supernatant was discarded, the pellet was resuspended in 25 mM HEPES, 1.5% Triton X-100 (BioRad), aprotinin (1200 TIU/l), and 2.5 mM PMSF and centrifuged for 50 min at 200,000 *g* to remove the insoluble material. The supernatant was then applied to a WGA column coupled to agarose (Miles-Israel). After washing with 25 mM HEPES buffer, pH 7.4, containing 0.05% Triton X-100, the bound material was eluted with 25 mM HEPES containing 0.05% Triton X-100 supplemented with 0.3 M N-acetylglucosamine dissolved in the same buffer.

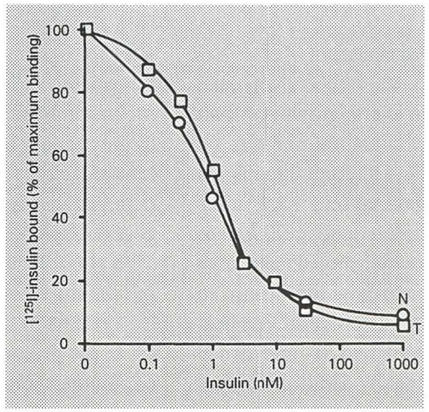


Figure 1 - [^{125}I]-insulin binding to normal (N) and tumor (T) colon tissue. WGA-purified insulin receptors were incubated with [^{125}I]-insulin and increasing concentrations of unlabeled insulin (0-1000 nM) for 45 min at 22°C. Binding of [^{125}I]-insulin is reported as percent of maximum binding obtained in the absence of unlabeled insulin. Each point represents the mean value of 5 normal and 5 colon tumor tissue samples. For all points the SEM was less than 5%.

Binding of [^{125}I]-insulin to WGA-purified receptor

Samples containing approximately 3 µg WGA-purified protein were incubated with 43 pM (20,000 cpm) [^{125}I]-insulin and various concentrations (0-1000 nM) of unlabeled insulin for 45 min at 22°C in a solution containing 50 mM Tris HCl, pH 7.5, 10 mM $MgSO_4$ and 1% bovine serum albumin (BSA). Separation of free and receptor-bound insulin was performed by using dextran-coated charcoal. The amount of [^{125}I]-insulin bound to the receptor was determined with a gamma-counter. Quantitative analysis of the Scatchard plots was performed according to the negative cooperative model (7) with a computer program (8).

Receptor phosphorylation

Similar amounts of WGA-purified receptor protein insulin (concentrations of 0-1000 nM) were preincubated at 21°C for 30 min. The material was immediately incubated with [gamma^{32}P]ATP (10 mCi/mM) (New England Nuclear, Germany) in an elution buffer containing 10 mM $MnCl_2$ and 1 mM vanadate at 21°C for 10 min. The incubation was stopped by the addition of Laemmli buffer containing 100 mM dithiothreitol and the samples were boiled for 15 min at 95°C. The phosphoproteins were separated by 7.5% polyacrylamide gel electrophoresis and identified by autoradiography. Labeled

bands were cut out of the gel and Cerenkov radiation was measured.

Data are reported as mean ± SEM. For statistical analysis, the Student *t*-test for dependent samples was used.

Results

Figure 1 shows the binding of [^{125}I]-insulin to partially purified membrane proteins from normal and tumor colon tissues. Half maximal displacement occurs at 1 nM unlabeled insulin in tumor and in normal tissues. The affinity of the purified insulin receptor for insulin was similar (Kd ≅ 1 nM) in normal and tumor tissues. Analysis of ligand binding of colon carcinoma and adjacent normal tissues yielded typical curvilinear Scatchard plots (Figure 2). These curves suggest high- and low-affinity binding sites for insulin or a model of negative cooperativity. Using equal amounts of WGA-purified proteins for binding studies, we found an approximately 1.7-fold increase in insulin receptors in tumor tissue (normal tissue: 17.4 fmol/µg and tumor: 29.69 fmol/µg). The Scatchard analysis of the binding data suggests unaltered affinity of the insulin receptor from carcinoma and normal tissue (high affinity), whereas the total amount of receptor protein is increased in tumor tissue (low affinity).

For autophosphorylation we diluted the partially purified insulin receptors on the basis of the Scatchard analysis to yield the same amount of insulin receptor. To measure autophosphorylation of the isolated insulin receptors, the sample eluates were incubated *in vitro* with [gamma^{32}P]ATP and various concentrations of insulin. The autoradiograms in Figure 3 show that insulin stimulates, in a dose-dependent manner, the incorporation of [gamma^{32}P] into the 95-kDa ß-subunits of the insulin receptor of both normal (Figure 3A) and tumor colon tissues (Figure 3B). Compared to normal tissue, [^{32}P] incorporation into the 95-kDa ß-subunit of the insulin receptor from carcinoma tissue is clearly increased at all insulin concentrations. The quantification of the dose-response curve from 5 colon carcinoma and 5 normal adjacent colons is shown in Figure 4. The insulin-stimulated incorporation of [gamma^{32}P] into the 95-kDa ß-subunits of normal and tumor colon reaches its half maximal effect at 1 nM and the maximal effect at 1000 nM, suggesting that the receptor sensitivity to insulin is not different in both tissues. The basal tyrosine kinase activity in the 95-kDa ß-subunit was 12.9 (% of maximum binding) in normal and 28.6 (% of maximum binding) in tumor (2.2 times higher in tumor). The insulin-stimulated tyrosine kinase activity was on average 1.6 times higher in colon tumor tissue, for each stimulated point of the curve (Figure 4).

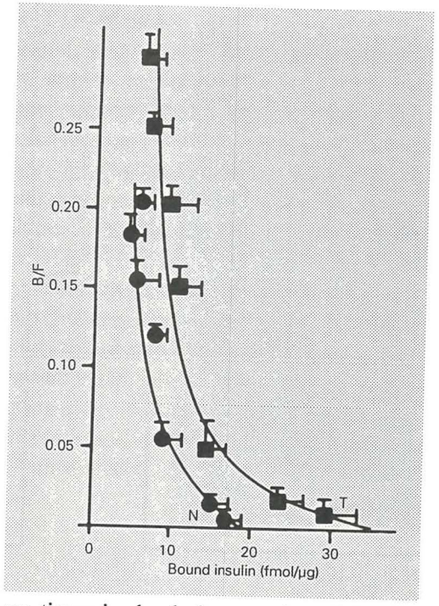


Figure 2 - Scatchard plot of the binding of [^{125}I]-insulin to WGA-purified receptors from normal colon (N) and colon carcinoma (T). Each point represents the mean value (± SEM) of 5 normal and 5 colon tumor tissue samples. Horizontal SEM, bound ligand; vertical SEM, bound to free ligand (B/F).

Discussion

Growth factors including insulin-like growth factor I (IGF-I) and IGF-II (9), EGF (10,11), TGF-α (11) and insulin (4) have been implicated in the proliferation of colon carcinoma cells. Koenuma et al. (3) have studied the effect of various growth factors

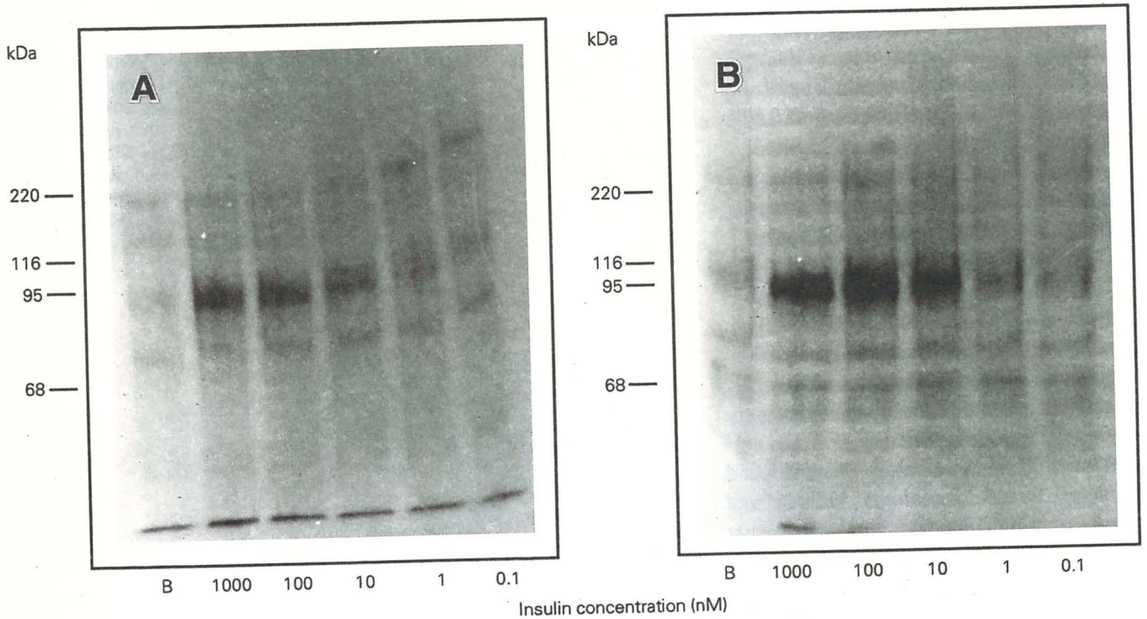
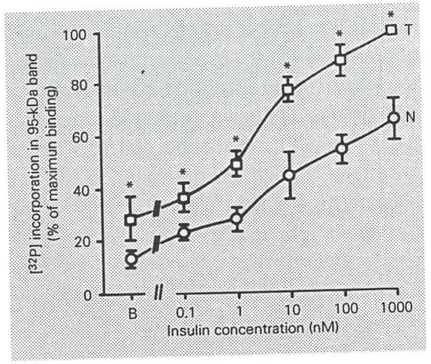


Figure 3 - Autoradiogram of dose-dependent autophosphorylation of WGA-purified insulin receptors from normal (A) and colon carcinoma (B) tissue. Partially purified receptor preparations were diluted on the basis of Scatchard analysis (Figure 2) to provide the same amount of receptor. WGA-purified receptor was incubated for 30 min at 21°C in the presence of BSA (B, basal) or insulin (0.1-1000 nM) and phosphorylated with [gamma^{32}P]ATP for 10 min at 21°C. Samples were subjected to 7.5% SDS-PAGE and the autophosphorylation of the 95-kDa ß-subunit of the insulin receptor was identified by autoradiography.

Figure 4 - Dose-response curve of [gamma^{32}P]ATP incorporation into the 95-kDa ß-subunit of the insulin receptors from normal (N) and tumor (T) colon tissue. The 95-kDa ß-subunit of the insulin receptor was identified by autoradiography (Figure 3), labeled bands were excised and [gamma ^{32}P]ATP incorporation was quantified with a beta counter. Data are reported as means ± SEM for five preparations of each type of tissue. B, Basal. *P<0.05 compared to normal colon tissue (Student *t*-test).



(EGF, MSA, IGF-I, TGF-α, IGF-II) and insulin in promoting growth of metastatic variants of colon carcinoma. This group showed that human insulin stimulates cell proliferation and DNA synthesis in these cells more than the other growth factors studied.

However, the mechanisms for the increased growth-promoting effect of insulin in carcinoma tissue are not known. The aim of the present study was to investigate whether this effect is determined by a quantitative aspect or by functional differences between the insulin receptors in colon carcinoma and normal colon. Our experiments were performed with partially purified receptors, which are subject to complications such as proteolysis and dephosphorylation (12). Receptors isolated from carcinoma and normal tissues had the same affinity for insulin, but in the carcinoma region of the colon, a clearly higher (1.7-fold) insulin receptor concentration on the cell membrane compared to the histologically normal colon region was observed. To compare the tyrosine kinase activity of the two tissues, we diluted the WGA eluate from colon carcinoma on the basis of Scatchard analysis to yield the same amount of receptors. After dilution, we still found a significant increase in basal as

well as insulin-stimulated tyrosine kinase activity of the insulin receptors isolated from colon carcinoma.

In breast tumor, Trischitta et al. (13) and Hilf et al. (14) found that insulin receptor tyrosine kinase activity of carcinoma tissue is similar to that of normal breast tissue. Kellerer et al. (15), comparing normal and neoplastic renal tissue *in vitro*, recently demonstrated that renal carcinoma expresses an elevated amount of insulin receptor protein with increased specific phosphorylation and tyrosine kinase activity. Although Koenuma et al. (3) and Watkins et al. (4) have found an increased growth-promoting effect of insulin from colon carcinoma compared to histologically normal colon, insulin receptors have not been studied in detail. The results of the present study suggest two explanations for the growth-promoting effects of insulin on colon tumors. The higher insulin receptor concentration in the carcinoma tissue offers more binding sites to insulin and probably causes an increased proliferation effect. Additionally, the increased tyrosine kinase activity in colon carcinoma may provide a more effective mechanism in the post-receptor signal transduction system. Whether this might be just an enhancing effect or whether the increased insulin receptor tyrosine kinase activity is also the key for a functionally altered signal transduction remains to be demonstrated.

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