Transferrin Receptors in Human Cancerous Tissues

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NIITSU, Y., KOHGO, Y., NISHISATO, T., KONDO, H., KATO, J., URUSHIZAKI, Y. and URUSHIZAKI, I. Transferrin Receptors in Human Cancerous Tissues. Tohoku J. exp. Med., 1987, 153 (3), 239-243 — The clinical significance of radioreceptor assay for transferrin receptors of human cancerous tissues was evaluated. Fresh surgical specimens from various carcinoma tissues were solubilized with 1% Triton X-100 and the extracts were mixed with $^{125}$I-labelled diferric transferrin. The free transferrin and the receptor-bound transferrin were separated by 15% polyethylene glycol precipitation. The % specific transferrin binding to gastric, colonic, lung and mammary carcinoma tissues ranged between 3.9 and 13.9%, whereas those for normal stomach and colon were less than 2%. The concentrations of transferrin receptors in these cancerous tissues ranged between 3.7 and 28.3 pmole/g tissues. It was concluded that the amounts of transferrin receptors were significantly increased in all of the tumor tissue extracts examined and may thereby provide a useful marker for the diagnosis of malignancies.

transferrin receptor; transferrin; radioreceptor assay; cell proliferation

The first event in cellular iron uptake is the binding of diferric transferrin to its receptors (Aisen and Listowsky 1980). In addition to erythroid cells (Jandl and Katz 1963), transferrin receptors (Tf•R) have been found in established cell lines in vitro (Hamilton et al. 1979; Trowbridge and Omary 1981). Recently, Shindelman et al. (1981) and Habeshow and Lister (1983) have reported the presence of Tf•R in the tissue of breast cancer and malignant lymphoma, respectively. Furthermore, it has become apparent that the Tf•R are expressed in much greater amounts on malignant cells than on non-malignant cells, although a quantitative study is lacking. These findings have generated a considerable interest on Tf•R which appear to be a marker of malignant transformation. In the present paper, we investigated Tf•R in surgical specimens obtained from a variety of different types of human malignancy. The results of our study indicate that Tf•R may be useful as a marker of cell proliferation.

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**MATERIALS AND METHODS**

*Triton extract of tissues*

Cancerous tissues from the stomach, colon, lung and breast were obtained surgically. Normal tissues for control studies were obtained from the healthy part of each specimen. The preparation of tissue extracts was done according to the method of Smith et al. (1977). Briefly, all tissues were homogenized in 10 mM Tris-HCl buffer, pH 7.4, immediately after the resection. The homogenate was centrifuged at 800 x g for 10 min and the supernatant was again centrifuged at 45,000 x g for 30 min. The pellet was washed with 10 mM citrate, pH 5.5, to remove pre-bound endogenous transferrin to the receptor (Kohgo et al. 1984). An extract of Tf-R from the pellet with 1% Triton X-100 in 10 mM Tris-HCl, pH 7.4, was performed for 1 hr at 4°C and the protein concentration of the extract was adjusted to 0.2 mg/ml (Wada et al. 1979).

*Transferrin*

Human serum transferrin was purified as described previously (Urushizaki 1983). Transferrin was radiolabeled with Na125I by lactoperoxidase method (van Renswoude et al. 1982). The specific activity of 125I-labelled transferrin was 10 μCi/μg.

*Radioreceptor assay*

The binding studies of 125I-transferrin with solubilized receptors in Triton extract from various tissues were carried out as follows (Wada et al. 1979). Radiiodinated transferrin (3 x 10⁶ cpm, 10 ng) was added to the aliquot of the extracts. The mixture was incubated for 30 min at 37°C, followed by the addition of polyethylene glycol (PEG) at the final concentration of 15% to precipitate the transferrin-Tf-R complexes. After incubation for 30 min, the mixture was centrifuged at 20,000 x g for 30 min at 4°C and the radioactivities of the pellet were determined by an autogamma counter (LKB, Wallac 1280, Sweden). The specific binding of 125I-labelled transferrin to Tf-R was obtained by subtracting the radioactivities of samples which had been incubated in the presence of unlabelled excess transferrin. The ratio between specific binding and the added total counts was calculated and expressed as percent bound. The amounts of Tf-R in 1 g tissue was calculated by the specific binding of 125I-transferrin to the pellets, the specific activity of 125I-transferrin described above and transferrin molecular weight 80,000. All assays were done in triplicate.

**RESULTS**

Pre-washing of the tissue homogenate with 10 mM sodium citrate buffer, pH 5.5, prior to radioreceptor assay enhanced the specific binding of 125I-transferrin by 20% approximately. In addition, the binding of 125I-transferrin to tissue extracts was saturable in 20 min at 37°C in time dependent manner. The maximum specific precipitation of transferrin-Tf-R complex was obtained at 15% concentration of PEG in the assay system. The results of radioreceptor assay performed with the extract of lung cancer and normal lung tissues are shown in Fig. 1. The total precipitated radioactivity was 22.4% and non-specific binding to the extract was 8.5% as determined by incubating in the presence of 1,000 ng of unlabelled transferrin. The difference of these two numbers were taken as a specific binding between 125I-transferrin and its receptor. On the contrary, there was little specific binding of 125I-transferrin to the extract of normal lung tissues.
The results of the radioreceptor assay from other tissues are summarized in Table 1. The specific binding of 125I-labelled transferrin to the extracts from normal tissues of stomach, colon and lung ranged between 0.6 and 2%. Cancerous tissues from stomach, colon, lung and breast showed a significant binding of 125I-transferrin ranging from 3.9 to 13.9%. The estimated amounts of the Tf•R in neoplastic tissue extracts ranged between 3.7 and 28.3 pmole/g tissue. On the other hand, control tissues possessed receptors less than 3.0 pmole/g tissue.

**DISCUSSION**

Previous studies on Tf•R in human neoplastic tissues have been made by immunohistochemical technique (Habeshow and Lister 1983; Shindelman et al. 1981), which could not provide quantitative data. This study shows that the content of receptors specific for transferrin in various cancerous tissues including the lung, stomach, colon and breast is substantially greater than that of normal conterpart. Lloyd et al. (1984) have described that only a small amount of Tf•R was observed either in normal of dysplastic epithelia of uterine cervical biopsy specimens by immunohistochemical study. We have found that the amount of Tf•R of hyperplastic nodules in the rat liver induced by diaminoaazobenzene was the same as that of the normal liver by 125I-transferrin binding study (unpublished

![Graph](image.png)

**Fig. 1.** Radioreceptor assay of transferrin receptors in the Triton extracts of lung cancer and the adjacent normal tissue.

A : $^{125}$I-transferrin precipitated in the absence of unlabelled transferrin.

B : $^{125}$I-transferrin precipitated in the presence of unlabelled transferrin (1,000 ng).

The data are expressed as a percent of radioactivity added to the incubation mixtures ($3 \times 10^6$ cpm), and the specific percent binding was taken as the difference between A and B.
Therefore, Tf • R exist in neoplastic tissue at high levels and most likely is reflective of malignant transformation.

In order to obtain the reliable data by radioreceptor assay using 125I-labelled transferrin to estimate tissue Tf • R, we washed the crude membrane fraction with 10 mM sodium citrate buffer, pH 5.5, a condition in which the pre-bound transferrin is dissociated from the receptor (Kohgo et al. 1984). Initial binding assays without citrate washing gave variable results and low levels of specific transferrin binding. This procedure increased the specific binding of 125I-transferrin to the extracts by 20% approximately.

Since the number of Tf • R on various tumor cell lines such as K562, M7609 and HL60 are greater than those of activated lymphocytes (Urushizaki 1983), Tf • R of infiltrating lymphocytes to cancerous tissues does not seem to contribute to the results of the radioreceptor assay significantly. The wide distribution and high content of Tf • R in neoplastic tissues indicate that the level of Tf • R may be a potential marker for diagnosis of malignancies and estimation of the proliferative activity of malignant tumor as suggested by Habeshow and Lister (1983), who pointed out that the Tf • R was valuable to estimate the grading of malignant lymphomas.

Although the present method was useful to carry out quantitative analysis,
the procedure is rather complicated in terms of the preparation of the samples and the determination of the optimal condition for the assay. The development of a direct quantitative assay such as radioimmunoassay for the receptor will simplify the procedure.

Another concern about Tf•R is whether there exists any qualitative difference in transferrin receptors between neoplastic tissues and their normal counterparts. The clarification of such differences will make it possible to use Tf•R as a more specific marker of the malignancies and a tool for therapeutic use.

References