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Expression of c-*kit* Receptor in Normal and Transformed Human Nonlymphoid Tissues[1]

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ABSTRACT

The protooncogene c-*kit* encodes a tyrosine kinase with a molecular weight of 145,000, highly related to the platelet derived growth factor/colony stimulating factor receptors. Mutations of the murine gene result in impairment of hematopoiesis, gametogenesis, and of the melanocyte cell lineage. In order to elucidate c-*kit* functions in development and oncogenesis we have analyzed immunohistochemically its expression in human normal and transformed nonlymphoid tissues. The receptor has been detected in spermatogonia, melanocytes, and unexpectedly, in astrocytes, renal tubules, parotid cells, thyrocytes, and breast epithelium. While the gene product is expressed in seminoma, lung tumors, and melanoma of low invasiveness, no detectable levels have been detected in thyroid and breast carcinomas, astrocytomas, and invasive melanomas. In breast tumors these findings were confirmed by paired, Northern blot analysis of RNA preparations from normal and transformed tissue. The present results demonstrate that the c-*kit* receptor plays a role in the development of a larger spectrum of cell lineages. Furthermore, on the basis of the transformation associated changes, we speculate that, while in some cell types, c-*kit* expression positively regulates mitogenesis and is selected for in neoplastic transformation, in other tissues the c-*kit* pathway is involved in morphogenesis and differentiation and is, therefore, negatively selected in the course of tumor progression.

INTRODUCTION

The c-*kit* protooncogene represents the cellular homologue of v-*kit* oncogene derived from the acutely transforming feline retrovirus HZ4-FeSV (1). The gene encodes a tyrosine kinase receptor which bears high homology with the platelet derived growth factor and colony stimulating factor receptors (2) and possesses several important roles in normal development which have been identified by mutations at the dominant white spotting (*w*) murine locus (3). Independent mutations which vary in their degree of severity affect, in fact, the development of hemopoietic cells, germs cells, and melanocytes (4). While a number of studies have analyzed at RNA levels the presence of c-*kit* gene transcripts in a limited number of normal (5), and tumor (6, 7) tissues, and during murine embryogenesis (8), little is known about the *in vivo* distribution of the gene product in normal and transformed human cells. Because this type of information, as it has been shown for other oncogenes products, may be of biological (9, 10), as well as of clinical (11, 12) relevance, in the present study we have addressed this issue by performing an extensive immunohistochemical analysis. This has been done by using monoclonal antibodies to the extracellular domain of the c-*kit* gene products on a variety of normal tissues and their transformed counterparts. The results of this study demonstrate that, differently from what has been so far reported, the c-*kit* product is unexpectedly expressed in a number of normal epithelia and undergoes different modulation patterns in pulmonary neoplasias, astrocytomas, seminomas, breast and thyroid carcinomas, and in malignant melanoma.

MATERIAL AND METHODS

Tissue Samples and Immunohistochemical Studies. Normal and neoplastic tissues were obtained from surgical samples of patients free from chemo- and radiotherapy. Tissues from brain, choroid plexus, and heart were obtained during autopsy. Each sample was divided into two parts: one was processed for conventional histopathological diagnosis, and the other was immediately snap-frozen in liquid nitrogen. Four-μm thick cryostat sections were stained with 0.1% toluidine blue in phosphate buffered saline (0.1 M sodium phosphate buffered saline, pH 7.2) and additional frozen sections were used for indirect immunofluorescence and immunoperoxidase staining after fixation in cold absolute acetone for 10 min. At least three nonconsecutive sections of the biopsy were analyzed. When possible, tissue obtained from more than one individual was tested.

The murine MoAb[3] 14A3 and 16A6 of the IgG2a isotype to the extracellular domain of c-*kit* were obtained by using NIH 3T3 fibroblasts transfected with a c-*kit* expression plasmid (2) as immunogen. The MoAb immunoprecipitate a single component with a molecular weight of 135,000–145,000 (13). The purified reagent (14) was used at concentrations ranging from 10 to 50 μg/ml. Indirect immunoperoxidase staining was performed with commercially available reagents (Immunocolor, Sorin Biomedica, Saluggia, Italy). Slides were incubated overnight with MoAb at 4°C in a moist chamber. The enzymatic activity was developed by using 3-amino-9-ethylcarbazole as chromogenic substrate for 8 min. Slides were then rinsed with phosphate buffered saline and counterstained with Mayer's hematoxylin. Sections on which the incubation of the primary antibody was omitted were used as controls.

Northern Blot Analysis. Total RNA was isolated from tissue samples according to the method of Chomczynski and Sacchi (15). Ten μg of each sample were analyzed on formaldehyde agarose (1.2%) gels as described by Lahrach *et al.* (16). The RNA was transferred to nitrocellulose filters and was hybridized to a radiolabeled *Eco*RI/*Apa*I restriction fragment (1312 base pairs) isolated from cloned c-*kit* complementary DNA (2). Filters were washed and exposed to X-ray film at −70°C for 5 days with the use of an intensifying screen (Cronex Lightning Plus). Computer assisted scanning of the gels was performed by using the McIntosh 2 program version 1.22 provided by Dr. Wayne Rusband (NIH, Bethesda, MD).

RESULTS

Distribution of c-*kit* Product in Normal Adult Human Tissues. Testing of normal tissues of various embryonic origin (Table 1) indicates that the product of the c-*kit* gene has a restricted tissue distribution. Detectable levels of the gene product were present, in fact, in epidermal melanocytes (Fig. 1*A*) at

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[3] The abbreviation used is: MoAb, monoclonal antibody.

Table 1 *Expression of c-kit gene product in normal adult human tissues*

Negative	Retina (1),[a] ciliary body (1), choroidal plexuses (1), hypophysis (1), peripheral nerves (2), lung (5), bronchus (2), liver (2), stomach (3), pancreas (2), gall bladder (1), jejunum (2), colon-rectum (4), epididymis (1), fallopian tubes (2), ovary (2), uterine cervix (2), urinary bladder (1), smooth muscle (2), placenta (2), keratinocytes (adnexa), spleen (2), prostate (1), seminal vesicles (1), thymic epithelium (2), endometrium (2), myocardium (1), adrenals (2).
Positive	Basal portion of renal distal tubules (3), acinar cells of parotid (2), spermatogonia (2), thyrocytes (2), melanocytes (6), alveolar and ductal cells of mammary epithelium (6), astrocytes (2), Purkinje cells (1), isolated small size vessels.

[a] Numbers in parentheses, number of individuals tested. Avidin/Biotin indirect immunoperoxidase staining.

all body sites, in large cells located along the basement membrane of the seminiferous tubules and in granular cells which were abundant in the submucosae. While, in most cases, the latter cells clearly displayed the morphological features of mast cells, in a number of instances their histological nature could not be firmly established. An occasional finding was the staining of the capillary walls. Additional tissue reactivities were the staining of (*a*) the basal portion of distal renal tubules, (*b*) the parotid acinar cells, (*c*) thyrocytes, and (*d*) alveolar and ductal breast epithelium (Fig. 1*B*) of astrocytes and Purkinje cells. In normal tissues the expression of the epitope identified by MoAb 14A3 was clearly associated with the cell plasma membrane. In all specimens tested with no detectable stain, the reactivity remained unchanged by using a higher concentration (50 μg/ml) of MoAb 14A3, prolonging the incubation time of the tissue substrates with the primary antibody, and using an equimolar pool of MoAb 14A3 and 16A6.

Changes in Expression of c-*kit* Oncogene Product in Human Solid Tumors. Immunohistochemical testing of a variety of primary solid tumors (Table 2) demonstrated that the product of the c-*kit* gene is expressed in a limited number of tumor histotypes. The receptor was found, in fact, in all seminomas (Fig. 1*C*), including dysgerminoma and gonadoblastoma and in all microcytomas tested, in 50% of the pulmonary adenocarcinomas, and less frequently in squamous and large cell tumors (Fig. 1*D*). On the contrary, in astrocytomas (18 cases tested), in breast (Fig. 1*E*), and in thyroid carcinomas a consistent lack of expression of the c-*kit* product was observed. Northern blot analysis of c-*kit* mRNA performed in eight paired tumor-normal tissue specimens indicated in all instances that the negative immunohistochemical reactivity correlates with a lack of the specific transcript. Two representative cases are shown in Fig. 2. Although the decrease of the receptor molecule to undetectable levels appeared to be independent from tumor histotype and degree of differentiation, in cutaneous malignant melanoma (Fig. 1*F*) a progressive decrease in reactivity with MoAb 14A3 was associated with increasing dermal invasiveness of the tumor (13). A low number of positive tumors were found in malignancies of the urinary bladder, ovary, and kidney. As an occasional finding, high levels of c-*kit* product were expressed in primary and metastatic tumors by unidentified interstitial cells and by small sized vessels (Fig. 1*E*).

DISCUSSION

Changes in the structure and level of expression of growth factor receptors are clearly implicated in the process of abnormal growth control and transformation (for review see Refs. 17 and 18). Their increasing identification in a number of malignancies provides information both on the molecular basis for their oncogenic potential and on their role in cell growth and differentiation (19, 20), and offers experimental models to define the intracellular pathways of signal transduction (21).

Furthermore, the structure of the growth factor receptor, characterized by a large extracellular domain mediating ligand binding and receptor-ligand internalization, makes them ideal targets in immunodiagnostic and therapeutic protocols which use monoclonal antibodies to either the cell surface functional domain or to the ligand. Using newly developed monoclonal antibodies to the extracellular domain of the c-*kit* protooncogene product, in the present study we have analyzed the tissue and tumor specificity of this gene product. The results of this study, derived from an extensive immunohistochemical phenotyping, have shown that the c-*kit* product displays a tissue distribution wider than that so far demonstrated from genetic and molecular biology analysis (4, 5). In fact, while c-*kit* has been confirmed in epidermal melanocytes and in germinal cells, low levels of expression of the receptor have been detected also in thyrocytes, in parotid acinar cells, and in some cases in the basal portion of distal renal tubules. Furthermore, high levels have been found in mammary epithelium. In contrast to what has been reported on RNA analysis performed on murine tissues, no c-*kit* product has been detected immunohistochemically in human placenta (4). This wider tissue distribution of c-*kit* in different epithelia further underlines the pleiotropic function (8) of this receptor molecule and raises the possibility that c-*kit* ligand interactions may also result in a spectrum of different cellular activities. The recent identification of the c-*kit* ligand (22–25) will undoubtedly facilitate the detailed investigation of these functions. Analysis of transformed tissues has demonstrated that c-*kit* undergoes different patterns of modulation. As reported by Strohmeyer *et al.* (7), at the RNA level we have detected c-*kit* receptor in all the seminomas tested but not in any of the embryonal carcinomas. Also of interest, the two cases of dysgerminoma and gonadoblastoma, which share the same histogenesis with seminoma (26), were found immunoreactive. At variance from what has been described by Sekido *et al.* (6), who identified c-*kit* transcript only in small cell lung cancer, we

Table 2 *Change in expression of the c-kit gene product in solid tumors*

Tumor from	Fraction positive
Breast (mixed histiotype)	10/80 (tumor cells and occasionally septa)
Thyroid (mixed histiotype)	2/14 (follicular carcinomas)
Lung (mixed histiotype)	22/49
Microcytoma	7/7
Adenocarcinoma	10/21
Squamous carcinoma	3/14
Large cell carcinoma	2/7
Testis	8/8
Seminoma	6
Disgerminoma	1
Gonadoblastoma	1
Embryonal carcinoma	0/3
Colon-rectum	0/19
Liver	0/11
Pancreas	0/3
Stomach	0/9
Endometrium	0/10
Skin (mixed histiotype)	0/15
Urinary bladder	3/10 (tumor cells)
Prostate	0/6
Ovary (mixed histiotype)	2/15 (tumor cells)
Brain (mixed histiotype)	3/36 (tumor cells)
Soft tissues (mixed histiotype)	0/11
Kidney	1/12 (tumor cells)
Adrenal	0/2
Melanocytes	22/31 (tumor cells)

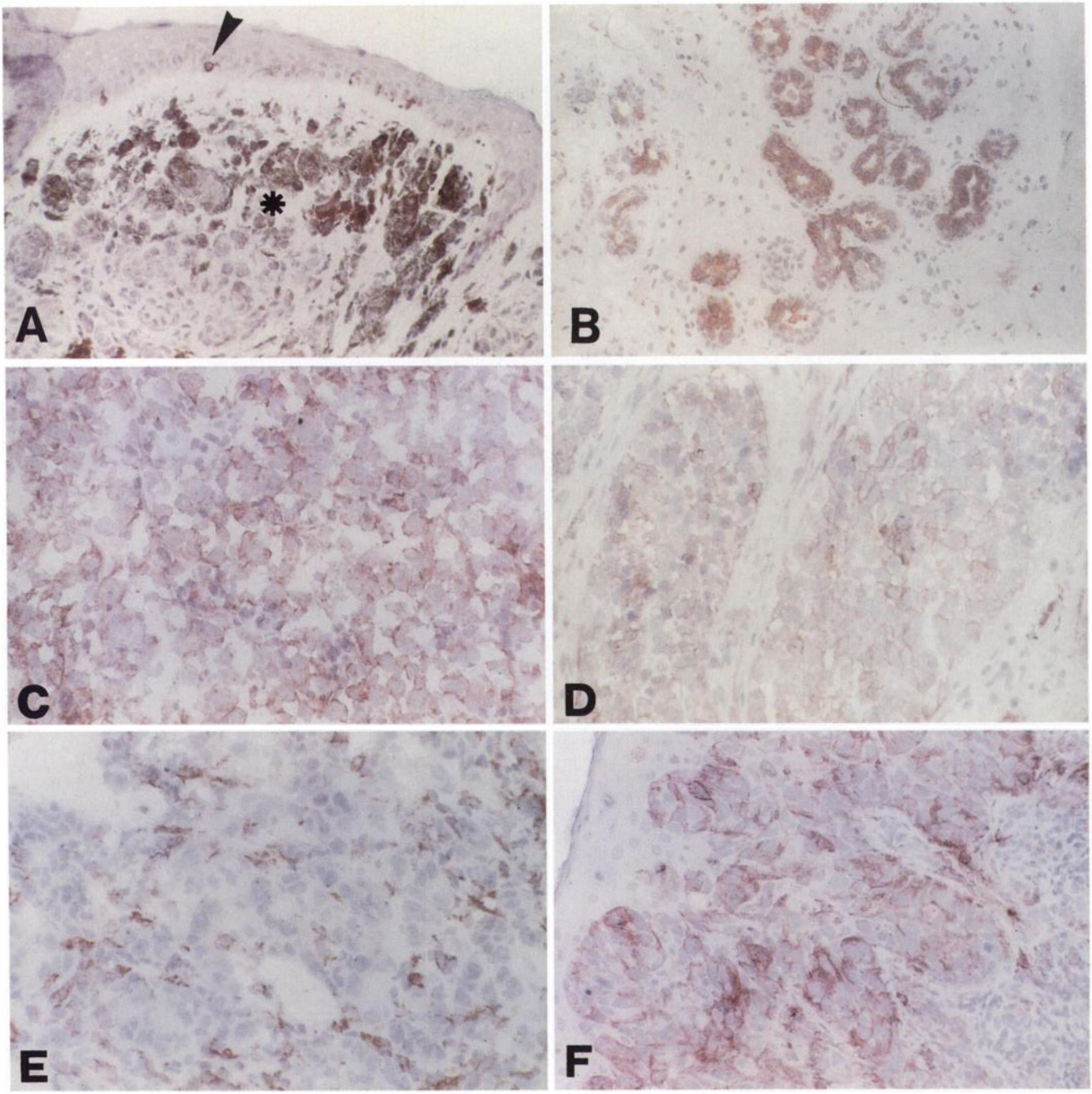

Fig. 1. Expression of the receptor encoded by c-*kit* protooncogene in normal and transformed human tissues as detected by indirect avidine-biotine immunoperoxidase stain using MoAb 14A3 on 4-μm cryostat sections. The antibody decorates homogeneously melanocytes (*arrow*) in the epidermis overlying an intradermal nevus (*) (*A*) and the plasmamembrane of normal mammary epithelium (*B*). The receptor is expressed by seminoma cells (*C*) and by an undifferentiated large cell lung carcinoma (*D*). No detectable levels of the gene product are expressed by cells of a breast carcinoma (*E*). A case of early invasive cutaneous melanoma displays heterogeneous levels of the receptor. In the mammary tumor some unidentified interstial cells are stained by MoAb 14A3. Mayer's hematoxylin counterstain. (*A*, *B*: *bar* = 20 μm; *C-F*: *bar* = 13 μm).

found that detectable levels of c-*kit* protein product are expressed also by other lung tumor carcinomas, thus indicating that c-*kit* expression does not reflect a unique biological feature of these neuroendocrine tumors. Nonetheless, c-*kit* receptor in small cell lung cancer may be of functional relevance in this malignancy which is influenced in its growth by autocrine and paracrine mechanisms (27). A completely different pattern of expression of c-*kit* has been found, on the other hand, in astrocytomas, thyroid carcinomas, mammary tumors, and in malignant melanoma. In these malignancies a significant decrease in c-*kit* gene product has been observed. Furthermore, in primary melanoma (13), these changes are clearly associated with the degree of tumor progression (28), *i.e.*, dermal invasiveness. Although the molecular basis of these changes is still incompletely defined, in some mammary tumors they can be correlated with the lack of RNA specific transcript. The apparent paradox of the conflicting pattern of expression of the c-*kit* receptor in tumors of different histiotype may be reconciled, considering

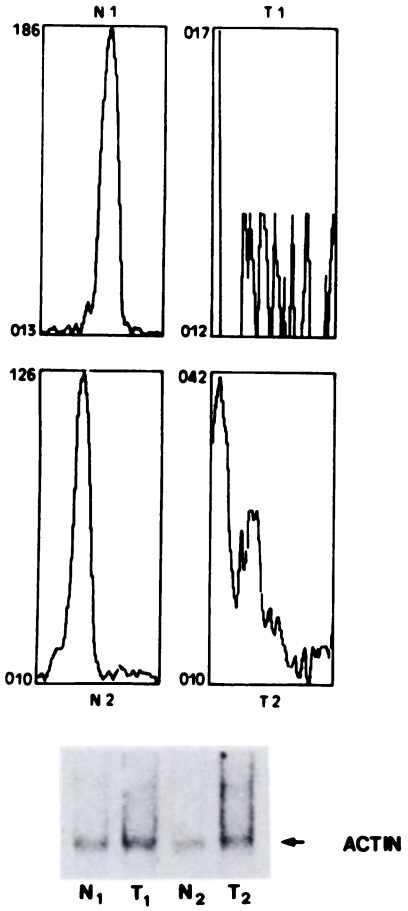

Fig. 2. Computer assisted image analysis of Northern blot analysis of total RNA isolated from two breast carcinomas (T_1, T_2) and from corresponding autologous normal breast epithelium (N_1, N_2). *Bottom*, hybridization patterns of the RNA samples with actin probe. The transformed samples display no measurable levels of the specific transcript. *Ordinate*, reported arbitrary densitometric values.

the dual effect of some oncogene products in different cell lineages. Thus, both v-*ras* and v-*src* oncoproteins can cause the differentiation of PC12 cells (29, 30), whereas they act as potent transforming genes in other cell types (31, 32). Likewise the c-*fms* product, which is the receptor for the colony stimulating factor, is important in regulating the survival and differentiation of mononuclear phagocytic cells (33), while behaving as a transforming protein upon transduction in fibroblasts (34). In this context our data suggest that the c-*kit* receptor may exert potent mitogenic effects in some cell types, thus undergoing positive selection during tumor progression. In contrast in other cell lineages, *i.e.*, melanocytes and mammary epithelium, the c-*kit* product may be important in regulating cell differentiation and tissue morphogenesis, and for this reason the loss of its expression may be a required step during tumorigenesis. The possibility of transfecting the c-*kit* gene in transformed cells of defined proliferative patterns and tumorigenicity may offer a model to investigate this issue. The finding that high levels of c-*kit* product could be occasionally expressed by cells of the tumor interstitium suggests that this receptor can be induced by environmental factors of still undefined nature. In conclusion, the present study has provided the experimental basis for future areas of investigation on the physiological role of c-*kit* in the differentiation of some epithelia and on its pathogenetic relevance in the onset and maintenance of the transformed state in a number of cell lineages.

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