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A Comparison of KGF Receptor Expression in Various Types of Human Cancer

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Abstract. *Background: Keratinocyte growth factor (KGF) has been observed to produce a rapid increase in the motility of breast cancer cells. KGF/KGFR (KGF receptor) signaling has also been demonstrated in the progression of many types of human cancer. The objective of the present study was to compare KGFR expression in various types of cancer. Materials and Methods: A cancer profiling array containing cDNA from 154 tumor and paired normal samples representing 19 types of human cancer was employed. Results: The results of the present study indicate that KGFR expression is enhanced in many types of human carcinomas at an early stage of cancer development, suggesting that KGFR overexpression may be an early signal in the progression of these cancers. However, the stage of cancer progression and relative level of expression varied considerably among the various types of cancer. Conclusion: These findings suggest that tumor KGFR levels may serve as a prognostic biomarker for cancer staging and/or treatment.*

Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor family (also designated FGF-7) that is produced in stromal tissue and stimulates DNA synthesis, proliferation and migration of epithelial cells in the breast and other tissues (1-3). It is well established that target epithelial cells contain a high affinity KGF receptor (KGFR) (4, 5). *In situ* hybridization studies have confirmed the specific mesenchymal production of KGF and epithelial localization of the KGFR in mammary tissue which provides further evidence that KGF is a mesenchymally-derived mediator of epithelial cell proliferation and migration (6, 7).

The mammary glands of adult female animals are remarkably sensitive to KGF (8). Systemic administration of KGF in adult rats for three to five days was found to produce massive mammary ductal hyperplasia and an elevation of mitotic figures (8). Intraductal hyperplasia is a well-known characteristic of pre-malignant breast lesions which lead to neoplasia. Similarly, Kitsberg and Leder (9) have observed that female mice, with a constitutively up-regulated KGF transgene, developed mammary epithelial hyperplasia and eventually all animals developed metastatic mammary carcinomas. Consistent with this concept, KGFR gene up-regulation has been observed in human primary breast tumor specimens (10). Conversely, highly malignant metastatic breast cancer tissue expressed relatively little KGFR (11). It has been observed that KGF treatment induced an up-regulation of the KGFR gene in estrogen receptor (ER)-positive breast cancer cells (12). These observations suggest that KGF-mediated stimulation of breast epithelial proliferation and migration may be an early event in the molecular cascade, which leads to cancer progression and metastasis (13). It has been previously determined that KGF produces a rapid, direct motility enhancement effect in ER-positive human breast cancer cell lines (14). Furthermore, KGF enhanced the growth of breast tumors in a mouse xenograft model (15). In addition to breast cancer, there is evidence which indicates that KGF/KGFR signaling is involved in the proliferation, invasion and malignancy of prostate (16-19), cervical (20), colorectal (21), ovarian (22), lung (23), stomach (24) and endometrial (25, 26) carcinomas. Therefore, KGF/KGFR signaling may be involved in the progression of many types of cancer.

The objective of the present study was to compare the expression of KGFR in cancer tissue relative to patient-paired normal tissue in order to determine the potential value of KGFR as a predictive biomarker of cancer metastasis and as a therapeutic target for the prevention of cancer progression.

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Table I. *Relative level of KGFR expression in human carcinomas.*

Cancer type	Breast	Ovary	Uterus	Cervix	Vulva	Prostrate	Testes	Thyroid	Skin
KGFR+	(6/10)	(10/10)	(7/10)	(3/10)	(1/5)	(3/4)	(8/10)	(1/10)	(0/10)
	4.56	1.07	2.99	0.66	0.33	1.18	1.51	0.31	0.63
	5.79	2.94	2.66	0.85	0.37	0.90	1.76	0.32	0.27
	0.57	4.10	1.21	0.63	0.53	2.31	1.47	0.50	0.40
KGFR	1.65	1.70	0.79	0.72	0.24	1.74	0.35	0.95	0.74
Ratio*	0.63	4.94	1.07	1.85	1.65		1.47	0.32	0.21
	0.59	1.92	0.92	1.61			3.48	1.15	0.50
	1.06	3.49	2.55	0.26			0.68	1.00	0.39
	0.49	1.30	0.61	0.61			2.76	0.28	0.46
	1.32	2.28	4.54	0.55			2.34	0.47	0.34
	1.29	1.35	3.69	2.37			2.75	0.86	0.59

Cancer type	Stomach	Small Intestine	Colon	Rectum	Pancreas	Liver	Lung	Kidney	Bladder
KGFR+	(6/10)	(5/7)	(1/10)	(4/10)	(2/7)	(0/3)	(6/10)	(1/10)	(2/5)
	1.46	0.96	0.48	0.84	0.72	0.25	1.34	0.34	0.88
	0.88	1.27	0.80	1.11	0.54	0.58	0.77	0.71	0.58
	34.91	0.67	1.24	3.58	1.30	0.23	0.75	0.47	1.17
KGFR	0.86	1.40	0.77	0.90	0.45		3.08	0.30	0.89
Ratio*	0.74	1.32	0.84	0.42	1.05	———	0.64	0.17	2.59
	1.31	2.33	0.90	1.98	0.25	Trachea (2/3)	2.23	0.19	
	4.35	1.24	0.94	0.78	0.45	———	1.04	0.58	
	41.17		0.40	0.91		1.76	4.65	0.23	
	1.42		0.87	1.00		0.87	0.38	0.94	
	0.82		0.55	1.07		1.36	2.65	1.01	

+Fraction of tumors with KGFR over-expressed relative to the patient-paired normal tissue within the cancer type subset. *Ratio of KGFR hybridization in tumor samples relative to the patient-paired normal tissue.

Materials and Methods

The CLONTECH Cancer Profiling Array II (BD Biosciences, Palo Alto, CA, USA) was employed in this study. This nylon membrane-based array was spotted with total cDNA extracted from 19 different cancer types and adjacent normal tissue taken from 154 individual cancer patients, according to the manufacturer's instructions. The membrane was also spotted with total cDNA from the following human cancer cells lines: *HeLa*, cervical carcinoma; *Daudi* and *Raji*, Burkitt's lymphoma; *K562*, chronic myelogenous leukemia; *HL60*, acute promyelocytic leukemia; *G361*, malignant melanoma; *A549*, lung carcinoma; *MOLT4*, acute lymphoblastic leukemia and *SW480*, colorectal adenocarcinoma. The array cDNA samples were normalized with ubiquitin.

The KGFR (FGFR2-IIIb) probe used in this study was created by isolating total RNA from an MCF-7 cell line using the RNeasy Mini-Kit (Quiagen, Valencia, CA, USA). The KGFR cDNA was directly prepared from 5 μg of total RNA by a SuperScript First-Strand Synthesis System for RT-PCR Kit (Invitrogen, Carlsbad, CA, USA). The polymerase chain reaction (PCR) for the probe was carried out using gene-specific primers: FGFR2-IIIb Reverse 5'-CAC TCG GGG ATA AAT AGT T-3' and FGFR2-IIIb Forward 5'-ACT CGG AGA CCC CTG CCA-3'. Gel electrophoresis of the final PCR product produced a single band with a size of 461 bp. An epidermal growth factor receptor (EGFR) Probe was created using the same methodology. The KGFR and EGFR cDNA probes were hybridized to the cancer array, as described in the manufacturer's protocol. Following hybridization, the arrays were placed on a phospho-imager (model 820 Storm Phospho-imaging System) overnight to create a digitized image of the array. The images were then analyzed using Array Vision software (Imaging Research Inc.) which calculates the pixel density of each of the elements (cancer and normal) and the background density. The element density is corrected for background density and the ratio of the corrected density of each cancer elements is divided by the corrected density of the patient-paired normal elements to obtain the hybridization ratios.

Results

The KGFR cDNA probe did not hybridize to the negative controls on the array which included total yeast RNA, *E. Coli* DNA, Poly A and a human DNA tandem repeat. The KGFR probe did hybridize to human genomic DNA used as a positive control. The ratio of the expression of KGFR in the tumor samples, relative to that present in the patient-paired normal tissue, is presented in Table I.

A ratio of greater than 1 indicates overexpression of KGFR in the cancer sample. In summary, the percentage of cancer samples which were observed to have an enhanced level of KGFR expression are as follows: breast 60%; ovary 100%; uterus 70%; cervix 30%; vulva 20%; prostate 75%; testes 80%; thyroid 10%; skin 0%; stomach 60%; small intestine 71%; colon 10%; rectum 40%; pancreas 29%; liver 0%; trachea 67%; lung 60%; kidney 10%; and bladder 40%. Breast tumor specimens with a higher KGFR expression were observed to be in an early stage of cancer development (stage I). This was found to be true in the other types of cancer except for ovary, stomach, rectum, bladder, trachea, small intestine and pancreas cancer samples, which were found to be in stages III or IV. All cDNA samples from the human cancer cell lines on the array were found to hybridize with KGFR and the density hybridization was similar among the cancer cell lines.

In this study the cancer array was also hybridized with an EGFR probe. It was observed that hybridization with the EGFR probe produced a pattern of tumor and tissue expression of EGFR which was completely different than that observed with the KGFR probe (data not shown). Thus, suggesting that the pattern of KGFR expression observed in this study was unique to KGFR.

Discussion

KGF binds to the KGFR (also known as FGFR2-IIIb) found in epithelial cells, which is a splice variant of FGFR-2 encoded by the FGFR-2 gene (27). KGFR is a member of the fibroblast growth factor receptor (FGFR) family which are membrane-spanning tyrosine kinase receptors consisting of four known peptides whose sequences are highly conserved (7). It has been shown that there is a transition in the KGFR from the IIIb isoform in primary tumors, which is KGF-responsive, to the IIIc isoform in advanced prostate cancer, which is unresponsive to KGF (19). This observation also supports the concept that KGF is an early signal that is involved in the initiation of cancer cell migration and progression to a metastatic phenotype.

It has been previously observed that KGF enhances the scattering motility of human breast cancer cells and that ER-positive, KGF-responsive cancer cells express KGFR while ER-negative, KGF-unresponsive, cells do not (14). Others have reported KGFR overexpression of *in situ* but not metastatic breast cancer (10, 11). The present study demonstrated that KGFR is up-regulated in breast and other types of cancer tissue at an early stage of cancer development (*i.e.*, uterus, cervix, vulva, prostate, testes and lung), suggesting that KGFR up-regulation may be an early signal in the progression of these cancers, and thus, may be a useful prognostic biomarker.

On the other hand, it was found that KGFR was up-regulated in more advanced tumors in other types of cancer (*i.e.*, ovary, stomach, small intestine, rectum, bladder, trachea and pancreas), while it was actually down-regulated in some other types of cancer on the array (*i.e.*, skin, liver, colon and kidney). These findings support the concept that KGFR tumor levels can serve as a prognostic biomarker for the staging and/or treatment of many types of cancer. Further, KGF, KGFR and associated signaling pathways may serve as therapeutic targets for the development of important new therapeutic agents to inhibit the metastatic progression of KGF-responsive cancers.

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