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Is LRIG1 a Tumour Suppressor Gene at Chromosome 3p14.3?

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Acta Oncologica Vol. 41, No. 4, pp. 352–354, 2002

The *LRIG1* gene (formerly *LIG-1*), recently cloned by us, displays structural similarities to the Drosophila *Kek 1* gene. *Kek 1* encodes a cell surface protein, Kekkon-1, which inhibits epidermal growth factor receptor-mediated signalling. We localized the *LRIG1* gene to chromosome band 3p14.3, a region known to be deleted in various human cancers. In the present study LRIG1 gene expression was examined in different tumour cell lines and corresponding normal tissues by real-time RT-PCR. In many tumour cell lines, LRIG1 expression appeared absent or was down regulated compared to corresponding normal tissues. The results are consistent with *LRIG1* being a tumour suppressor gene in humans. However, further studies are justified to elucidate the explicit role of LRIG1 as a negative regulator of oncogenesis.

Received 4 June 2002
Accepted 6 June 2002

Recently, we have cloned and sequenced an mRNA for human leucine-rich repeats and immunoglobulin-like domains 1 (LRIG1; formerly LIG-1), determined the chromosomal localization of the gene and analysed the expression in various human tissues (1). The gene is the human homologue of mouse Lrig1 (2) and encodes a transmembrane protein which, in the extracellular part, shows structural similarities to the Drosophila cell surface protein Kekkon-1 (3). The extracellular part of Kekkon-1 functions in Drosophila as an inhibitor of epidermal growth factor receptor (EGFR)-mediated signalling (4). The gene was localized to chromosome band 3p14.3, a region known to be deleted in various human cancers, including breast and lung carcinomas (5, 6). We have also developed antibodies specific for LRIG1 and observed a high expression in the epithelium of prostate, breast, lung and colon, tissues from which the majority of human cancers originate. The study confirmed our previously reported RT-PCR results from different human non-neoplastic tissues (1). Together with these observations, we found it of interest to report that LRIG1 appears to be down regulated in cell lines derived from lung, colon and prostatic carcinomas.

MATERIAL AND METHODS

Cell lines

The prostatic carcinoma cell lines PC-3 and DU 145 and the colorectal adenocarcinoma cell lines HT-29 and DLD-1 were obtained from American type culture collection (Manassas, VA, USA). The lung cancer cell line U1690 was kindly provided by Dr J. Bergh (Uppsala University, Sweden) and the lung mesothelioma cell line P31 by Dr B. Sandström (FOI, Umeå, Sweden). The cells were cultivated in DMEM supplemented with 10% foetal bovine serum and gentamicin, 50 μg/ml. Cell culture media and reagents were from Life Technologies AB (Täby, Sweden).

RNA and quantitative RT-PCR analysis

Total RNA from normal human tissues was purchased from Origene Technologies, Inc. (Rockville, MD, USA). RNA from the cell lines was prepared using the RNAqueous kit (Ambion Inc., Austin, TX, USA). RNA samples were DNase treated using the DNAfree kit (Ambion Inc.) prior to the real-time RT-PCR analyses. LRIG1 and 18S rRNA transcript levels were quantified by real-time RT-PCR, as previously described (1). Briefly, triplicate samples of 20 ng RNA were run and relative quantification was performed by comparing the threshold cycle values (Ct) for the samples with standard curves generated with the cloned cDNAs of respective genes. To correct for differences in RNA quality and quantity, the LRIG1 values were normalized to the apparent 18S rRNA levels in respective RNA samples.

 ISSN 0284-186X

RESULTS

To evaluate the LRIG1 mRNA expression levels in a panel of human carcinoma cell lines and their corresponding normal tissues, we used a quantitative real-time RT-PCR assay, as previously described (1). LRIG1 transcript levels were clearly lower in many of the carcinoma cells analysed compared to corresponding normal tissues (Fig. 1). This was especially evident in the P31 mesothelioma (Fig. 1A), PC-3 prostate carcinoma (Fig. 1B), and DLD-1 colorectal carcinoma (Fig. 1C) cell lines. In DLD-1, no expression at all was evident. In the prostate and prostate carcinoma cell lines, the expression pattern was confirmed by Western blot (data not shown).

DISCUSSION

In this study, using quantitative RT-PCR, we found that the *LRIG 1* gene was underexpressed in many tumour cell lines of epithelial origin compared to normal tissue. The down-regulation was especially evident in the mesothelioma cell line P31, the prostatic carcinoma cell line PC-3, and in the colorectal carcinoma cell line DLD-1. LRIG1 shows structural similarities to Kekkon-1 (1), which functions as a repressor of EGFR-mediated signalling in the fruit fly Drosophila melanogaster (4). Since EGFR-mediated signalling is deregulated in many human cancers, and since LRIG1 is localized to a chromosomal region, 3p14 (1), which is frequently deleted in a variety of human cancers (5, 6), we propose that LRIG1 might be a human tumour suppressor gene.

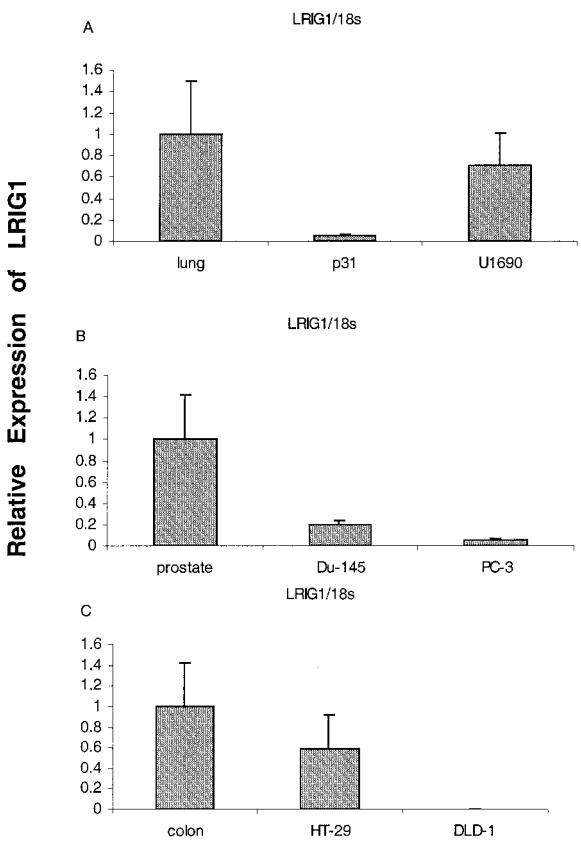

Fig. 1. Relative LRIG1 mRNA levels in normal tissues and cancer cell lines from lung (A), prostate (B) and colon (C). Quantitative real-time RT-PCR was performed using total RNA from respective tissues and cell lines. Shown are the relative LRIG1/18S ratios in respective samples. Error bars indicate the standard deviations of triplicate LRIG1 samples, and the means of triplicate 18S samples.

EGFR is a protein tyrosine kinase overexpressed in many types of tumour cells, including lung-, colon and prostatic carcinoma, and there is an association between up-regulation of the EGFR and poor clinical prognosis (7, 8). EGFR-signal transduction is regulated by stimulatory and inhibitory inputs. The well-studied activation of EGFR is associated with receptor phosphorylation and stimulation of pathways leading to cell proliferation and survival of tumour cells (7). Potentially inhibitory pathways are, however, markedly less well investigated. Kekkon-1, which participates in the inhibition of EGFR-mediated signalling in the fruit fly, displays structural similarities to LRIG1 (1, 4). Although the molecular function of LRIG1 is not known, it is tempting to propose that LRIG1 might act as a suppressor of signalling mediated by the EGFR or other growth factor receptors. Thus, the observed down-regulation and/or deletion of LRIG1 in the tumour cells analysed by us could potentially lead to disappearance of de-activating regulatory signals and constant autocrine activation of, for example, the EGFR, with subsequent cell proliferation and survival of tumour cells.

However, although the majority of neoplasms with 3p14 deletions are epithelial in origin, it is suggested in a previous report that the mouse LRIG1 homologue is predominantly expressed in the brain (2). This would seem contradictory to LRIG1 being a tumor suppressor gene associated with 3p14 deletions. Recently, we developed antibodies specific for LRIG1 and analysed their expression in normal human tissues using immunohistochemistry and Western blotting (Nilsson et al., manuscript). This study confirmed our previously reported RT-PCR results (1) showing that human LRIG1 expression is not restricted to the brain. In particular, we noted prominent expression in the epithelium of prostate, breast, lung and colon, the tissues from which the majority of human cancers originate. Hence, taken together, the present observations that LRIG1 is missing or down regulated in tumour cells, and previously published data, are compatible with LRIG1 functioning as a tumour suppressor in human epithelial neoplasms. Nevertheless, future studies are necessary to address the molecular function of LRIG1 and its role in the development of different cancers.

ACKNOWLEDGEMENTS

This study was supported by grants from the Lions Cancer Foundation, Umeå, and the Swedish Cancer Society, Sweden.

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