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LATS2 tumour specific mutations and down-regulation of the gene in non-small cell carcinoma

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ABSTRACT

LATS2 is a new member of the LATS tumour suppressor family. The human LATS2 gene is located at chromosome 13q11-12, a hot spot (67%) for loss of heterozygosity (LOH) in non-small cell lung cancer (NSCLC). We screened 129 non-small cell lung cancer samples and 13 lung cancer cell lines, initially for mutations in the LATS2 gene and subsequently for mutations in P53 and K-RAS genes. Either polymorphisms or mutations were identified in over 50 percent of analysed tumours. A novel missense mutation, S1073R, and a large deletion of 8 amino acids in the PAPA-repeat region were detected in 9 and 2 NSCLC tumours, respectively. Those mutations were not identified in the 13 lung cancer cell lines. Mutations were tumour specific and were absent from adjacent normal tissue and healthy controls. Down-regulation of the LATS2 gene was observed in most NSCLC tumours but was not related to any mutation or polymorphism. Tumours with a LATS2 mutation often also harbour a P53 but not K-RAS gene mutation and were mostly in an advanced stage of development, with regional lymph node involvement.

1. Introduction

The whole LATS tumour suppressor family plays an important role in the control of tumour development and cell cycles [1–3]. Two homologs, LATS1 and LATS2, are functionally conserved and regulate the cell cycle progression and apoptosis [1,4,5]. LATS1 is implicated in the regulation of the cell cycle at the G2/M [2,3] and LATS2 at the G1/S phases [6,7]. LATS1 is thought to be a tumour suppressor gene, since LATS1 deficient mice develop soft tissue sarcomas or ovarian stromal cell tumours [4], and LATS2 also to be a tumour suppressor gene, since frequent allelic imbalances at 13q have been reported in various human cancers, also in NSCLC, where LOH has been found in over 60% of tested tumours [8].

The C-terminal regions of LATS1 and LATS2 contain the putative Ser/Thr protein kinase domain and closely resemble each other (84.6% of similarity in catalytic domains), whereas the sequence similarity of the N-terminal region is very low [5,6]. Both genes are also components of the very complex Hippo signalling pathway, which plays a key role in organ size control by regulating cell proliferation and apoptosis [9]. It has been proposed that Lats1/2 induces phosphorylation and inhibition of the YAP (Yes-associated protein), and regulates cell contact inhibition [9,10]. The main difference between the LATS2 and LATS1 genes is the absence of the SH3 binding motif in LATS2, which is found in LATS1. A difference in distribution patterns also seems to be of particular importance. LATS1 is expressed at considerable levels in the brain, liver and kidney, where LATS2 is scarce [1]. Data revealing differences in treatment response relating to the levels of mRNA expression of the homologs in breast, testis cancers, leukemias and astrocytomas, may show differences of involvement of the two genes in tumourigenesis and be used for the prediction of prognosis and response to chemotherapy treatment [11–14].

The LATS2 gene mapped on 13q11-q12 encodes the 1046-amino acid protein, which contains a PAPA repeat, consisting of seven copies of dipeptide proline-alanine, which may be involved in protein–protein interactions, and a C-terminal serine-threonine kinase domain [5]. The role of LATS2 was demonstrated on a mouse model in 2004, when it was concluded that LATS2 has a role in maintenance of mitotic fidelity and genomic stability, because LATS2−/− mutant embryonic cells exhibited an increased frequency of cytokinesis defects, accumulation of micronuclei, supernumerary centrosomes and aneuploidy [15,16]. Another report revealed the LATS2 function as inducer of apoptosis through down-regulation of anti-apoptotic proteins of the Bcl-family [17]. In 2006, the putative function of LATS2 became clearer with the report by Aylon et al. [18]. The findings implicate LATS2 as the key mediator

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of the G1 tetraploidy checkpoint, while LATS2 translocates into the nucleus by mitotic apparatus dysfunction and inactivates Mdm2 [18]. It was demonstrated that LATS2 interacted physically with MDM2 to inhibit p53 ubiquitination and to promote p53 activation and, in contrast, down-regulation of LATS2 compromised the p53 mediated G1 tetraploidy checkpoint. LATS2 itself is a target for positive transcriptional regulation by p53, and the authors of the study concluded that the LATS2-MDM2-p53 pathway is critical for the maintenance of the proper chromosome number [18]. According to recent publications, LATS2 is involved in many other pathways, with different roles in them. It has been implicated in the induction of p53-dependent senescence as a response to Ras activation [19] as well as in a novel pathway in which, by interacting with Ajuba, LATS2 modulates centrosome modulation [20].

Frequent polymorphic changes but rare tumour specific mutations of the LATS2 gene have been identified in esophageal squamous cell carcinoma [21]. However, until now no tumour specific mutations in the LATS2 gene have been reported in any type of cancer and to our best knowledge only 2 mutations (G40E and V925V) have been discovered in lung cancer [22] and 2 mutations in aorta endothelial tissue (A324V and G363S, reported in NCBI database under accession cDNA reference AB209897).

In our study, we evaluated the expression of LATS2 mRNA in non-small cell lung tumours and examined the relation between LATS2 and MDM2 expression, given that both genes are involved in the p53-LATS2 and MDM2 pathway. With screening of the LATS2 coding region and a portion of the 3′ UTR region we wanted to determine whether there are alterations specific for NSCLC and to examine putative relations between P53, LATS and K-RAS mutations.

2. Materials and methods

2.1. Tumour samples and DNA preparation

Altogether, 129 surgical specimens of patients with primary non-small cell lung carcinoma tumours (51 adenocarcinoma, 67 squamous cell carcinoma, and 11 large cell carcinoma) were included in our study. Lung resectates were examined by an expert pathologist. Adjacent macroscopically normal lung tissue was obtained several cm from the tumour whenever possible and used as a control. All tumours were histologically evaluated and snap-frozen in liquid nitrogen and stored at −70 °C until used. The study included 107 (83%) men and 22 (17%) women patients with ages ranging from 42 to 83 years (mean ± S.D.; 65.4 ± 9.1 years). The following 13 lung cancer cell lines were also included in the study: NCI-H23, NCI-H226, NCI-H322M, NCI-H460, NCI-H522, HTB178, HTB182, H466, H1299, Calu1, Calu3, SkMes-1 and A549. One hundred healthy control DNA samples, consisting of 79 male (79%) and 21 female (21%) blood donors, with a mean age of 39.8 years (S.D. ± 7.1 years), were used to evaluate LATS2 mutations and polymorphisms in a normal population.

The National Medical Ethics Committee of the Republic of Slovenia (NMEC) approved the study. DNA was extracted from snap frozen lung cancer tumours and corresponding normal tissue using NucPrep™ chemistry (Applied Biosystems, Foster City, CA, USA) on an ABI 6100 (Applied Biosystems, Foster City, CA, USA). DNA from the control blood samples was extracted using a QIAamp DNA Blood Midi Kit™ (Qiagen, Germany).

2.2. Screening of the LATS2

LATS2 mutational status was determined by mutation analysis on DHPLC (Transgenomic, USA). Suitable newly designed primers for DHPLC detection were chosen with an online oligo-designer (IDT® SCITools Oligo Design and Analysis), and the conditions for DHPLC mutation screening were selected according to Wavemaker™ (Transgenomic, USA). Screening of the whole gene was done by appropriate sets of primers (Table 1). Samples with confirmed distinct elution chromatograms were subsequently sequenced to determine the exact nature of the alterations.

DHPLC temperatures used for profiling were chosen according to the Wavemaker™ program to screen the whole amplicon and were

EX1 [UTR]: 61 °C; 66.7 °C; 69 °C
EX2 [UTR+EX]: 53 °C; 54 °C; 60.5 °C; 61.5 °C
EX3: 61.7 °C; 62.7 °C
EX4-1: 64.5 °C; 66.6 °C
EX4-2: 59.2 °C; 60.5 °C
EX4-3: 50 °C; 67 °C; 68 °C; 69 °C
EX4-4: 59.2 °C; 60.7 °C
EX5: 57.8 °C; 58.2 °C; 60.2 °C
EX6: 55.2 °C; 56.7 °C; 63.2 °C
EX7: 61.4 °C; 63.4 °C
EX8-1: 60.8 °C; 62.2 °C
EX8-2: 53.8 °C; 57.3 °C

The concentrations of WAVE Optimised buffers™ A, B, D and Wave syringe solution were default for mutation detection, as the flow and time of elution, were calculated with the Wavemaker program™. In order to obtain heteroduplexes the PCR products were denatured and renatured and then injected into a DNASep cartridge (Transgenomic Inc., Omaha, NE, USA) and eluted at a flow rate of 0.9 ml/min through a linear gradient of acetonitrile containing 0.1 M triethylammonium acetate (TEAA). The elution gradient of Buffer A (0.1 M TEAA solution) and Buffer B (0.1 M TEAA containing 25% acetonitrile solution) was automatically adjusted using Wavemaker™ software (Transgenomic Inc., Omaha, NE, USA). For DHPLC of the P53 gene, primers and conditions were used as described for esophageal cancer [23]. For DHPLC screening of K-RAS mutational hotspots in codons 12, 13 and 61, primers were used as already described by Konig et al. [24], and the column temperature was calculated with the Wavemaker program™.

Table 1
Primers used for genomic DHPLC analysis, oligonucleotide sequences of PCR primers, PCR product lengths and annealing temperatures.

Exon	5′ Forward primers	5′ Reverse primers	Length (bp)	Annealing *T* (°C)
EX1	GCCCGTGGAATGCCAACAAT	CGACCGCTTCGCGGAAACTCT	307	63
EX2	AGGTGATGCGTTCTTTCT	TAGTTGCCAAACCATTCT	754	52
EX3	GCCACCTGCACTCATTCT	AAACCATCTTTGCCCACTA	335	55
EX4-1	ACTCTGATGCTGTCTTCCTGTT	GGGGTGGGAAAGCGAGGC	479	60
EX4-2	TCCTTCCAGAGCAAGACGC	ACACGCACGCTCTTCACC	527	61
EX4-3	TGCACCCGGTGAAGAGCGTG	GTGCTTCCTATTGCCAGTAG	643	57
EX4-4	GGACAAAGGCGGAAAGGAT	AAAGGCGAAAGGGAGGTCA	400	55
EX5	TAGGCGACAGAGCGAGAC	AGCCACTAGGATGGGAAA	839	53
EX6	TGTCAGGAGCTGATTGGA	AGGAGGGAGGCTTGTATG	471	53
EX7	GTAGGGATTGAGTGAACTT	GATGTCTACAGCAAGCAG	177	51
EX8-1	ACTAACCTGTTGGCTCTG	TGAAGTAATCGACGGACT	648	54
EX8-2	AAGCCGAGGGCTGTTTTG	CTCCGTGACATTGAGCAGAGTG	395	50