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C to A single nucleotide polymorphism in intron 18 of the human MST1R (RON) gene that maps at 3p21.3

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Abstract

The MST1R (RON) gene, that maps at 3p21.3, encodes a protein tyrosine kinase receptor comprised of an extra-cellular domain that contains the ligand binding pocket and an intracellular region where the kinase domain is located. It controls cell survival and motility programs related to invasive growth. With the single strand conformation polymorphism (SSCP) method, a C to A nucleotide polymorphism (SNP) was found in intron 18 of the gene. The SNP has a frequency of 0.28 among African–American, 0.25 among Caucasian CEPH and 0.09 among Asian healthy individuals. During these studies, an alternatively spliced cDNA of MST1R, lacking exon 19, was also found that may result from this change.

Keywords: Alternative splicing; Breast cancer; MST1R (RON); Center d'Etude du Polymorphisme Humain (CEPH); Single nucleotide polymorphism (SNP); Single strand conformation polymorphism (SSCP)

1. Introduction

The macrophage stimulating 1 receptor, MST1R (RON, OMIM; 600168) gene maps at 3p21.3, a region critical for breast and lung cancer [1]. MST1R protein signaling can be abnormally activated through several mechanisms. The experimental introduction of tumorigenic point mutations found in MET and KIT activates the oncogenic potential of RON [2]. The mouse and avian orthologs of RON (*stk* and *sea*, respectively) are involved in oncogenesis through the participation of viral component [3,4]. Rare splicing variants found in gastric carcinomas and cell lines lead to aberrant dimerization and constitutive activation of RON [5].

However, so far RON has been found to be involved in naturally occurring human tumors through over-expression. This is the case of breast and colon cancer. In normal breast epithelium and benign lesions, the protein is expressed at a very low level but the expression level increases significantly in infiltrating carcinomas [6]. Similarly, colon carcinoma cell lines show a high level of expression and constitutive activation of RON whereas normal epithelial colon cells do not [7,8]. Moreover, Ron was found highly expressed also in a number of non-small cell lung cancer [9] and activated in bronchioalveolar carcinomas (Danilkovitch et al., personal communication).

Using the single strand conformation polymorphism (SSCP) method we performed mutation analysis of several breast, renal and lung cancer samples in which the RON gene and the surrounding genomic region were found amplified (Zabarowsky et al., manuscript in preparation). During these studies a new single nucleotide polymorphism (SNP) was found: a C to A transversion that occurs 10 bp upstream of the acceptor splice site of intron 18 (GenBank entry: AF164652). The SNP frequency was evaluated in three different ethnic groups. An alternatively spliced cDNA

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lacking exon 19 was also found, along with the wild type cDNA, in a lung cancer cell line with the polymorphism.

2. Materials and methods

2.1. Human DNA

Signed, informed consent was obtained from all participants in the study, according to the NCI institutional review board-approved protocol.

2.2. Cell lines

Lung cancer cell lines NCI-H358, NCI-H1650, NCI-H1666 and NCI-H1781 were obtained from ATCC (Manassas, VA).

2.3. PCR primers

Primers were synthesized by ABI Applied Biosystems (Frederick, MD).

in 18 Fw: 5′ TTC CAG CTG AAG GAC TCT GG
in 19 Rv: 5′ ACT ACC ACC TCC ACA TAC TC
ex 18 Fw: 5′ ATC CTG GAC AGG GAG TAC
ex5 Rv: 5′ TCC CCA AGC AGT GCA GAC ACT ATC TG

2.4. PCR–SSCP analysis

The radioactive labeling reaction was performed in a total reaction volume of 12.5 μl, containing 100 ng of genomic DNA, 12.5 pmol of each primer (in 18-Fw, in 19-Rv), 200 μM dNTPs, 1.5 mM $MgCl_2$, 1.25 nCi α^{35}S-dATP. Primers amplified a single band (255 bp) under the following cycling conditions: 3 min at 95 °C; (30 s at 95 °C, 30 s at 60 °C, 30 s at 72 °C) for 35 cycles; 7 min extension at 72 °C. After heat denaturation (8 min at 90 °C) in formamide buffer (Stop Solution, Amersham, Arlington Heights, IL), PCR products were run overnight in a 0.5 × MDE gel (FMC Bioproducts, Rockland, ME), 0.6 × TBE, at room temperature, 8 W constant power; transferred on 3 mm paper, dried and exposed to auto-radiography film (X-OMAT AR, Kodak, Rochester, NY).

Table 1
Frequency of the SNP in three different ethnic groups

Ethnic group	Genotype			Frequency of allele A
	CC	CA	AA	
African–American	15	5	1	0.28
Asian	18	2	0	0.09
Caucasian (CEPH)	19	8	0	0.25

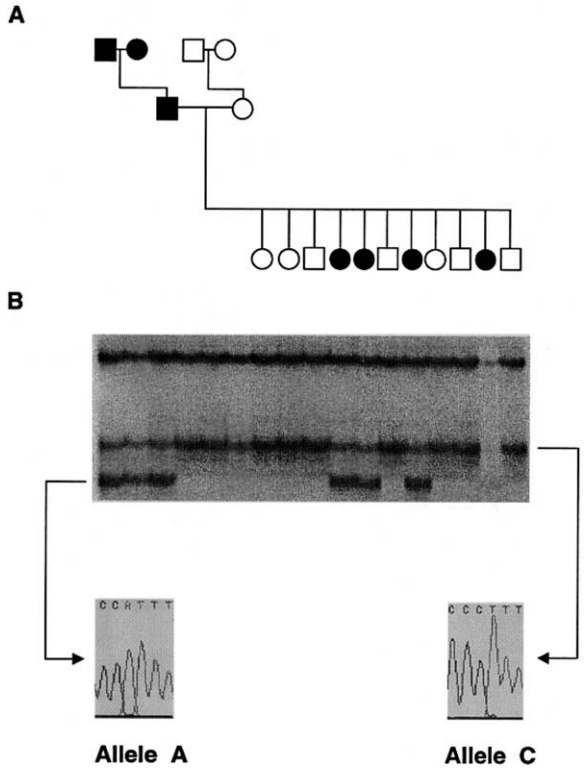

Fig. 1. SSCP analysis of the CEPH Family 1362 [10] shows Mendelian inheritance of the C to A SNP located in intron 18 (GenBank entry: AF164652) of the MST1R gene. (A) pedigree of Family 1362. (B) SSCP profile of allele 'C' and allele 'A' and corresponding sequence of subcloning products.

2.5. RT-PCR

First strand cDNAs were reverse transcribed from random primed total RNA isolated from cell lines above (cDNA Cycle Kit, Invitrogen, Carslbad, CA). MST1R cDNA was amplified with the primers ex18-Fw, ex5-Rv (PCR product: 356 bp) under the following conditions:

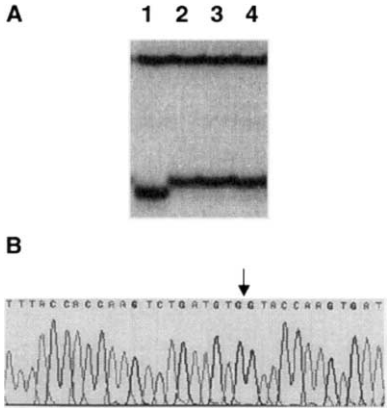

Fig. 2. Alternative splicing of exon 19 is present along with wildtype splicing in the SNP-carrier cell line H1650. (A) SSCP analysis of gDNA. (1) H1650 ('AA'), (2) NCI-H358, (3) NCI-H1781, (4) NCI-H1666 (all 'CC'). (B) Sequence of the alternatively spliced cDNA. The arrow points to the splice junction between exons 18 and 20.

3 min at 95 °C; (30 s at 95 °C, 30 s at 62 °C, 30 s at 72 °C) for 35 cycles; 7 min extension at 72 °C.

2.6. Sequencing

PCR products were cloned into the pCR2.1-TOPO vector (TA Cloning Kit, Invitrogen, Carslbad, CA) and several single colonies were isolated and sequenced.

Sequencing reactions were performed on an ABI 373 Stretch Automated DNA Sequencer (Applied Biosystems, Foster City, CA).

3. Results and discussion

A C to A transversion was found 10 bp upstream the acceptor splice site of the MST1R gene intron 18 (nucleotide 414 GenBank entry AF164652), it introduces a new XcmI site at base 92 of the 255 bp PCR product (Section 2). Three different ethnic groups were analyzed to establish the frequency of allele A in the general healthy population (Table 1). One carrier family (CEPH Family 1362 [10]) was analyzed to prove Mendelian inheritance of the genotypic trait (Fig. 1).

To investigate a possible effect of the SNP on the splicing process, we analyzed MST1R cDNA from cell lines homozygous for the 'A' (NCI-H1650), 'C' (NCI-H358, NCI-H1781 and NCI-H1666) alleles (Fig. 2A). Amplification of NCI-H1650 cDNA with PCR primers designed across exon 19 (ex18Fw, ex5Rv) yielded two bands (data not shown): the expected 356 bp product with wildtype sequence and a less abundant, alternatively spliced 219 bp product, lacking exon 19 (Fig. 2B). Under the conditions used the alternatively spliced cDNA was not amplified from cell lines with the C allele.

Because this polymorphism affects exon 19, a part of the MST1R kinase domain, we plan to test the hypothesis that it may lead to a predisposition for cancer.

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