Neurogenetics (2004) 5:81–82
DOI 10.1007/s10048-003-0164-x

LETTER TO THE EDITORS

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Mutation analysis of *CBL-C* and *SPRED3* on 19q in human glioblastoma

Published online: 14 November 2003

Keywords CBL-C · SPRED3 · Human glioblastoma

Sirs,

Allelic loss of 19q13 is one of the common molecular abnormalities in malignant gliomas such as glioblastoma. However, extensive mapping efforts and mutation screening of candidate genes at 19q13 have failed to identify a putative tumor suppressor gene in this region [1].

Alterations of receptor tyrosine kinase (RTK) signaling are also common in human glioblastoma. Amplification and overexpression of the *EGFR* (epidermal growth factor receptor) gene, encoding an RTK with known oncogenic function, is found in about one-third of glioblastomas, but it remains possible that activation of the EGFR signaling pathway occurs by other mechanisms in those glioblastomas that lack gene amplification. RTK signaling is partially controlled by negative regulators; these negative regulators are involved in cellular homeostasis and their deregulation occurs in human oncogenesis [2]. Two RTK negative regulatory genes map to 19q13: *CBL-C* [3] and *SPRED3* [4]. CBL-C is a member of the CBL family, which regulates EGFR activation by internalization and degradation via ubiquitination, thereby inhibiting EGF-stimulated MAPK activation. An alternative spliced form of CBL-C, which deletes a critical region of the phosphotyrosine binding (PTB) domain, abolishes interaction with and inhibition of EGFR [3]. RING finger mutation of CBL-A is also associated with EGFR activation [5]. SPRED, on the other hand, inhibits the EGFR-mediated RAS-MAPK pathway via an interaction with RAS-RAF [6]. SPRED3 contains an Ena/Vasodilator-stimulated phosphoprotein (VASP) homology-1 (EVH1) domain and Sprouty-related cysteine-rich region (SPR domain), but lacks a functional c-Kit-binding domain (KBD) [4]. Interestingly, *SPRED3* is expressed exclusively in the brain [4].

We hypothesized that genetic alteration of these genes could activate the EGFR signaling pathway in an alternative manner to *EGFR* amplification. To address this possibility, we performed single-strand conformation polymorphism analysis of the crucial, conserved domains of each candidate using the primers listed in Table 1. We screened the 8 exons of the PTB and RING domains of *CBL-C* and the 3 exons encoding the EVH1 and SPR domains of *SPRED3* in 30 glioblastomas (8 with *EGFR* amplification and 22 with normal *EGFR* copy number). One aberrant shift was detected in exon 8 of *CBL-C*, but this was also present in corresponding blood DNA. No somatic mutation was detected in either gene.

We conclude that neither *CBL-C* nor *SPRED3* is likely to be the 19q13 glioblastoma gene. Nonetheless, alterations of negative RTK regulatory molecules may play a role in human malignancies, including glioblastoma, and further study of these genes may be of interest.

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Table 1 Primers used for polymerase chain reaction/single-strand conformation polymorphism analysis of the *CBL-C* and *SPRED3* genes

Exon	Domain	Forward primer	Reverse primer
CBL-C			
1-1	PTB	GGCTCCCATGGCTCTGGCGGT	CGCTGTGCGGGGCAGCAGGT
1-2	PTB	GCTGTCCGTGAGTCCCCCTT	GGCCTCCAGATTGGCCAGGT
1-3	PTB	CGGCTCTGGGGACTTTCTAC	GAGCAAGACCTGGGCCTCAC
2	PTB	GGGAGCCCCAAGGATAGCCA	GCTCCGGGAGCTGAGGACAA
3	PTB	GTGTCTCCCCCACCCCTCTC	AGCTCCCAGCCTTGGCCTTC
4	PTB(SH2)	TCCCTGACCCAAGCCCTGCC	GATCCCTGAGCCCTGCAGCC
5	PTB(SH2)	ACTCCCTCACCCCATCCTAC	GCTGACACCCTCCTCCTACC
6	PTB(SH2)	CTGGGGGTGGGAAATACTGG	TCAGGGAACTGGGACTGCGG
7	RING	TGCCCCTCGCTGTCTTCTCT	CCTTTTGGGGCTTTCCCTGT
8	RING	CTTTCCCTCCCGACCTCCCC	CTCCAGTCCCTCCCCACTCA
SPRED3			
2	EVH1	TCCCTGTCCTTCCCCCCACC	CTACCCTCCCCGCATGCCCC
3	EVH1	CTGCTTTCCTTCTGCCCCTC	ACATCACCTGGGCTGCTCAC
6-1	SPR	TACCCTCCGCTTCTACCGTTCA	CCCCTCAACGCGCCGGTCT
6-2	SPR	CCCGGAAGCGGAGGAGGCAG	CAGGCGCACGGGTCCGAGAA
6-3	SPR	GCTTGCTCTACCACTGCCTGTC	GTCCTCACCGCGCAGCCTC

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