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Chk2 splice variants express a dominant-negative effect on the wild-type Chk2 kinase activity

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ABSTRACT

While the majority of RNA transcripts from protein-encoding genes in the human genome are subject to physiological splicing, pathological splicing is increasingly reported in cancer tissue. Previously, we identified >90 different splice variants of *Chk2*, a gene encoding a serine/threonine kinase propagating the DNA damage signal by phosphorylating and activating several downstream substrates like p53, Cdc25A, and Cdc25C involved in cell cycle arrest and apoptosis. While alternative splice forms of other genes have been reported to exert a dominant-negative effect on the wild-type molecules, the function of Chk2 splice protein variants is still unclear. Here we evaluated the function of four Chk2 splice proteins for which mRNA splice variants were identified in human breast carcinomas. These splice variants were stably expressed as nuclear proteins. Two splice forms (Chk2Δ4 and Chk2del(2-3)) expressed kinase activity while variants Chk2Δ11 and Chk2isoI were essentially kinase inactive. Independent of intrinsic kinase activity, each splice variant impaired wild-type Chk2 activity through heterodimerization. Based on our findings, we suggest alternative splicing as a possible novel mechanism for repression of the Chk2 wild-type function.

1. Introduction

Recent findings have proposed 60–80% of human genes to be subject to alternative splicing [1,2], a molecular mechanism providing protein diversity and suggested to regulate gene functions and activities. While splicing is a normal physiological event subject to regulatory factors [1,3], dysregulated splicing has been linked to various diseases including cancer [3–8]. In cancer cells, aberrant splicing may act oncogenic through inactivation of tumor suppressors, or, alternatively, by gain of function of novel proteins with tumorigenic activity [6]. For instance, it is well documented that isoforms of Kruppel-like factor 6 (KLF6), a transcription factor acting as a tumor suppressor, may antagonize the wild-type gene function [9].

Chk2 (checkpoint kinase 2) is a tumor suppressor and plays a key role in the cellular response to DNA damage. Activated ATM phosphorylates Chk2 at T68 located in the SQ/TQ domain [10], and subsequently, the SQ/TQ binds to the FHA domain of a second Chk2 molecule forming a dimer [11]. Dimerization promotes multiple autophosphorylation events including T383, T387, and S516 within the kinase domain [12,13]. Activated Chk2, which might persist in dimers or dissociate to monomers, then propagates the DNA damage response signal by activating downstream targets such as p53, Cdc25A, and Cdc25C [14–19].

Previously, we revealed >90 different splice variants of *Chk2* mRNA in breast cancer tissue [20], all expressed concomitantly with the *Chk2* wild-type transcript. Alternatively spliced *Chk2* forms were found also in non-tumor tissue samples, but the breast cancers harbored a higher number of splice forms in a more complex and heterogeneous splicing pattern. While functional analysis of six splice variants revealed lack of kinase activity among four splice forms and one was found to localize pan-cellularly [20], the biological role of *Chk2* splicing still remains poorly understood.

To study the function of Chk2 splice variant proteins and their potential interference with Chk2 wild-type function, four *Chk2* mRNA splice variants observed in human breast carcinomas [20] were selected based on their lack of sequences known to be essential for Chk2 function (Fig. 1). Most of the *Chk2* mRNA splice variants originally detected presented similar exon losses [20], thus, the splice forms studied in this report were chosen to represent the majority of the total *Chk2* splice repertoire observed in breast cancer biopsies. Notably, previous work from our group has shown defects in the Chk2-p53 pathway to be associated with resistance to anthracycline/mitomycin therapy [21–23]. Hypothesizing splice variants to express

Abbreviations: Chk2, checkpoint kinase 2; Chk2wt, Chk2 wild-type; FHA domain, fork-head associated domain; KD, kinase dead; LFS, Li–Fraumeni syndrome; RF, reading frame; SCD, SQ/TQ cluster domain; *TP53*, p53 tumor suppressor gene

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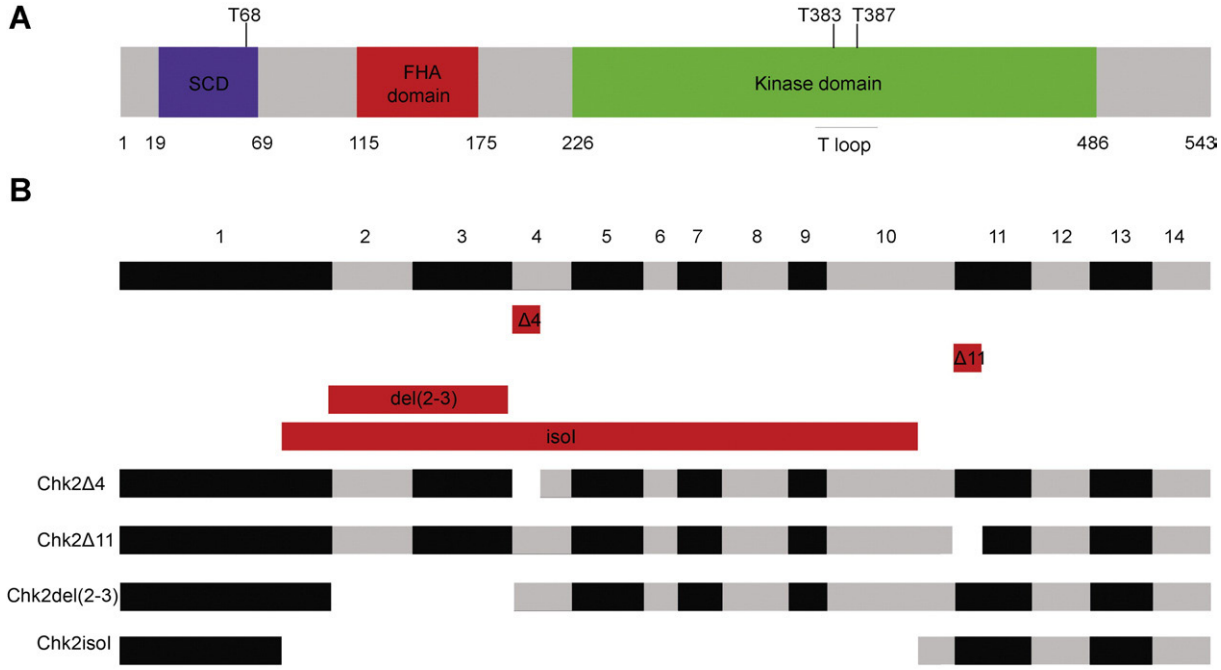

Fig. 1. Schematic presentation of Chk2wt and splice variants. (A) The Chk2 protein contains 543 amino acids and has three distinct functional domains; SQ/TQ cluster domain (SCD) harboring the T68 residue phosphorylated by ATM, fork-head-associated (FHA) domain which is a phosphopeptide recognition motif, and kinase domain including the T loop. (B) Overview of the 14 coding exons in *Chk2*. The regions missing in the four splice variants are indicated in red bars and white gaps.

antagonistic functions against Chk2 wild-type, we selected splice variants expressed in tumors resistant to doxorubicin therapy despite harboring wild-type *Chk2* and *TP53*.

Two of the four splice variants, *Chk2iosI* (GenBank accession number AY551295) and *Chk2del(2-3)* (GenBank accession number AY551303) were reported in our previous publication [20], while the other two, *Chk2Δ4* (GenBank accession number not yet available) and *Chk2Δ11* (GenBank accession number not yet available), were identified from the same material but have not been described previously. We here present data regarding the stability, subcellular localization, dimerization ability, and kinase activity of these splice variants, as well as their impact on the kinase activity of wild-type Chk2. All splice forms encoded stable Chk2 protein variants which localized to the nucleus and formed heterodimers with wild-type Chk2. Most importantly, they significantly reduced Chk2 kinase activity when co-expressed with the wild-type protein, thus revealing a dominant-negative effect on Chk2 wild-type.

2. Materials and methods

2.1. Plasmid construction

Among the 90 different splice variants of *Chk2* observed by Staalesen et al. in primary breast cancer tumors [20], *Chk2isoI*, *Chk2del(2-3)*, *Chk2Δ4* and *Chk2Δ11* were selected. The *Chk2Δ4* splice form analyzed in this work (GenBank accession number not yet available) should not be confused with Chk2del4 (GenBank accession number AY551302) reported in our previous work [20]. Detailed characteristics of the analyzed Chk2 splice variants are provided in Fig. 1 and Table 1. The PCR products of *Chk2* wild-type and splice forms were TA-cloned into the expression vectors pcDNA3.1/V5-His TOPO and pcDNA4/HisMax TOPO (Invitrogen, CA, USA), providing respectively C-terminal V5- and N-terminal Xpress tags. Primers for PCR and sequencing are listed in Table 2. The resulting constructs were named *Xpress-Chk2wt*, *Xpress-Chk2Δ4*, *Xpress-Chk2Δ11*, *Xpress-Chk2del(2-3)*, *Xpress-Chk2isoI*, *Chk2wt-V5*, *Chk2Δ4-V5*, *Chk2Δ11-V5*, *Chk2del(2-3)-V5*, and *Chk2isoI-V5*.

2.2. Cell culture

HCT116 (Chk2−/−) cells, from which the *Chk2* gene has been deleted by homologous recombination [24], and the parental cell line HCT116 were both kindly provided by Dr. F. Bunz (Johns Hopkins University). U-2 OS cells expressing only low levels of endogenous Chk2 [19,21], were purchased from ATCC. All three cell lines were cultured in McCoys 5A supplemented with 10% FBS. The MCF-7 cell line was routinely maintained in RPMI-1640 supplemented with 10% FBS and 2 mM L-glutamine. Transfection was performed using Fugene 6 (Roche, Mannheim, Germany) and Lipofectamine 2000 (Invitrogen)

Table 1
Characteristics of the analyzed Chk2 protein splice variants.

Chk2 protein variant	Chk2isoI	Chk2del(2-3)	Chk2Δ4	Chk2Δ11	Chk2wt
Missing basepairs	223–1176	320–592	593–619	1260–1292	
Reading frame after splice	In RF	In RF	In RF	In RF	
Conserved domains	SCD	SCD, kinase	SCD, FHA, kinase	SCD, FHA	SCD, FHA, kinase
Molecular mass (kDa) including V5 tag	32.4	59.6	69.5	69.2	70.6
Localization	Nuclear	Nuclear	Nuclear	Nuclear	Nuclear
Km (μM)	KD	0.87	0.07	KD	0.13
Vmax (pM/min/mg lysate)	KD	9.84	334	KD	156
Vmax/Km		11.3	4771		1200

Abbreviations: RF, reading frame; SCD, SQ/TQ cluster domain; FHA, fork-head associated domain; KD, kinase dead.