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CHES1/FOXN3 interacts with Ski-interacting protein and acts as a transcriptional repressor

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Abstract

Checkpoint Suppressor 1 (*CHES1*; *FOXN3*) encodes a member of the forkhead/winged-helix transcription factor family. The human *CHES1* cDNA was originally identified by its ability to function as a high-copy suppressor of multiple checkpoint mutants of *Saccharomyces cerevisiae*. Accumulating expression profile data suggest that CHES1 plays a role in tumorigenicity and responses to cancer treatments, though nothing is known regarding the transcriptional function of CHES1 or other FOXN proteins in human cells. In this report, we find that the carboxyl terminus of CHES1 fused to a heterologous DNA binding domain consistently represses reporter gene transcription in cell lines derived from tumor tissues. Using a cytoplasmic two-hybrid screening approach, we find that this portion of CHES1 interacts with Ski-interacting protein (SKIP; NCoA-62), which is a transcriptional co-regulator known to associate with repressor complexes. We verify this interaction through co-immunoprecipitation experiments performed in mammalian cells. Further analysis of the CHES1/SKIP interaction indicates that CHES1 binds to a region within the final 66 hydrophobic residues of SKIP thus defining a new protein–protein interaction domain of SKIP. These data suggest that CHES1 recruits SKIP to repress genes important for tumorigenesis and the response to cancer treatments.

Keywords: SKIP; Forkhead; Winged-helix; Checkpoints; Transcription factors

1. Introduction

Forkhead genes encode a large family of transcription factors, which contain a ~110 aa DNA binding domain known as the forkhead/winged-helix motif (Kaufmann and Knochel, 1996). The *Drosophila* gene *fork head* was the first characterized member of the forkhead family (Weigel et al., 1989) and over 100 family members have since been identified throughout eukaryotes. Phylogenetic analysis of the conserved DNA binding motif categorized forkhead members into 17 subfamilies, *FOXA* through *FOXQ* (Kaestner et al., 2000).

Forkhead proteins play diverse roles in vertebrate development and there is increasing evidence for forkhead involvement in the pathogenesis of multiple diseases (Lehmann et al., 2003). Mutations in several human forkhead genes, including *FOXC1*, *FOXC2*, *FOXE3*, and *FOXL2*, give rise to ocular disorders. Mutations in *FOXE1* result in a wide array of cranio-pharyngeal phenotypes and disruptions in *FOXP2* are associated with severe speech and language development disorders.

Accumulating data also indicate a role for forkheads in cell cycle regulation and tumorigenesis. The *Saccharomyces cerevisiae* Fkh1 and Fkh2 forkhead proteins are expressed in late S-phase to cooperatively regulate expression of a group of genes required for the G2-M transition, including

Abbreviations: CHES1, Checkpoint Suppressor 1; cDNA, DNA complementary to RNA; SKIP, Ski-interacting protein; aa, amino acid; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; RT, reverse transcriptase; DBD, DNA binding domain; ts, temperature-sensitive; kDa, kilodaltons; VDR, vitamin D receptor; RCC, renal cell carcinoma.

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SWI5, *CLB2*, and other genes necessary for mitosis (Zhu et al., 2000). The mammalian FOXM1 protein is induced during S-phase to insure its proper timing and prevents re-initiation of DNA replication before mitosis (Korver et al., 1997, 1998). FOXO1, FOXO3, and FOXO4 mediate cell cycle regulation in mammalian cells and over-expressing the forkheads induces expression of the cell cycle inhibitor $p27^{kip1}$ leading to cell cycle arrest in G1 (Medema et al., 2000).

Checkpoint Suppressor 1 (*CHES1*; *FOXN3*) encodes a 490 aa human forkhead protein of unknown function. In addition to *CHES1*, the *FOXN* subfamily contains five additional members: (1) *FOXN1* (*WHN*), which regulates keratinocyte growth and differentiation of thymic epithelium (Coffer and Burgering, 2004), (2) *FOXN2* (human T-cell leukemia virus enhancer factor), which binds to the human T-cell leukemia virus long terminal repeat and might be involved in its transcriptional regulation (Li et al., 1992), and (3–5) *FOXN4–FOXN6*, which were recently identified through bioinformatic approaches (Katoh and Katoh 2004a,b,c). The *FOXN* genes remain an understudied forkhead subfamily with little known about the transcriptional activity of the proteins within this group.

CHES1 was first isolated in a screen aimed at the identification of human checkpoint cDNAs by demanding high-copy suppression of checkpoint mutations in *S. cerevisiae* (Pati et al., 1997). The *CHES1* clone isolated in the screen contained cDNA encoding the final 200 aa of the protein and therefore lacked the forkhead DNA binding motif located near its amino-terminus (N-terminus). When exogenously expressed in checkpoint-deficient yeast mutants, the truncated cDNA increased survival in response to various forms of DNA damage and restored a DNA damage-induced G2 arrest. Subsequent work by our laboratory revealed that CHES1 binds in vivo to the yeast Sin3 protein, a component of the Sin3/Rpd3 histone deacetylase complex (HDAC) (Scott and Plon, 2003). Our data indicate that CHES1 inhibits the Sin3/Rpd3 HDAC and deletion of either *SIN3* or *RPD3* mimics those checkpoint phenotypes observed by expression of *CHES1* in checkpoint-deficient yeast strains.

Several groups have published data suggesting that regulation of *CHES1* might be important in the response to cancer treatments and the development of multiple tumor types, including renal cell carcinoma, oral squamous cell carcinoma, and epithelial ovarian cancer (Sawiris et al., 2002; Hong et al., 2003; Struckmann et al., 2004; Chang et al., 2005). Furthermore, *CHES1* under-expression was identified as being predicative of a positive clinical response in patients undergoing immunotherapy treatment for melanoma metastases (Wang et al., 2002).

This present study was initiated to gain insight on CHES1's function in human cells. We find that the carboxyl terminus (C-terminus) of CHES1 functions as a transcriptional repressor. Furthermore, we find that CHES1 binds to a novel protein–protein interaction domain on Ski-Interacting Protein (SKIP; NCoA-62), which is a transcriptional co-regulator capable of recruiting activation and repression complexes to DNA-bound transcription factors.

2. Materials and methods

2.1. Plasmid constructs

The *CHES1* two-hybrid bait vector *pSOS-CHES1* contains the *Not*I-restricted fragment of the *CHES1* cDNA corresponding to the C-terminus (aa 293–490; GenBank U68723) fused to the gene encoding human Son-of-Sevenless (hSOS) in vector *pSOS* (Stratagene). The two-hybrid control vectors, *pSOS-MAFB* and *pMYR-MAFB*, were obtained from Stratagene.

KS269 (full-length *CHES1* cDNA in *pBluescript II*) was digested with *Hind*III, followed by Mung Bean nuclease treatment and subsequent digestion with *Xba*I. The resulting fragment containing full-length *CHES1* was inserted into the *CS2MT* vector prepared by *Xba*I/*SnaB*I digestion to yield an N-terminal MYC-tagged *CHES1* fusion construct, *pMYC-CHES1*.

SKIP cDNA was amplified from a human cDNA library using primers 5′ATAAGAATGCGGCCGCTAAACTATATATGGCGCTCACCAGCTTTTTA and 5′CGGGATCCCGCTATTCCTTCCTCCTCTTCT. The product was confirmed by sequencing and digested with *Not*I/*Bam*HI for ligation into the corresponding sites in *pFLAG-CMV-2* (Sigma) to create the N-terminal FLAG-SKIP fusion construct, *pFLAG-SKIP*.

SKIP deletion clones *pMYR-SKIP-BclI* and *pMYR-SKIP-BstXI* were created by digesting the two-hybrid prey clone *pMYR-SKIP-1A* with *Xho*I/*Bcl*I and *Xho*I/*Bst*XI, respectively, followed by Mung Bean nuclease treatment and religation to remove cDNA distal to the *Bcl*I and *Bst*XI restriction sites. *pMYR-SKIP-Hydro* was created by PCR amplifying cDNA from *pMYR-SKIP-1A* using primers 5′CCGGAATTCCGGAACAGATTTGTTCCCGACAAGGAG and 5′CCGCTCGAGCGAGTTCATTCACTTTGGAGAGACCTG. The product was confirmed by sequencing and digested with *Eco*RI/*Xho*I for ligation into the corresponding sites in *pMYR* (Statagene). *pMYR-SKIP-SNW* was created by PCR amplification of *pFLAG-SKIP* using primers 5′CGGAATTCGCCATGGCGCTCACCAGCTTTTTACC and 5′CGGGATCCCGCTATTCCTTCCTCCTCTTCT. The product was confirmed by sequencing and digested with *Eco*RI and *Eco*RV for ligation into *pMYR*, which was prepared by digestion with *Sma*I, Mung Bean nuclease treated, and subsequent digestion with *Eco*RI.

pDNR3-tx23 contains the *Xho*I–*Xho*I fragment with *CHES1*'s C-terminus (aa 292–490) from *p424-GPD-CHES1* as described (Scott and Plon, 2003). *pDNR3-tx23* was digested with *Sac*I/*Eco*RI and the resulting fragment was ligated into *pFA-CMV* (Stratagene) to create the GAL4 DNA binding domain (DBD) fusion construct, *pDBD-*