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Fez1/Lzts1 Absence Impairs Cdk1/Cdc25C Interaction during Mitosis and Predisposes Mice to Cancer Development

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SUMMARY

The *FEZ1/LZTS1* (*LZTS1*) protein is frequently downregulated in human cancers of different histotypes. *LZTS1* is expressed in normal tissues, and its introduction in cancer cells inhibits cell growth and suppresses tumorigenicity, owing to an accumulation of cells in G2/M. Here, we define its role in cell cycle regulation and tumor progression by generating *Lzts1* knockout mice. In *Lzts1*$^{-/-}$ mouse embryo fibroblasts (MEFs), Cdc25C degradation was increased during M phase, resulting in decreased Cdk1 activity. As a consequence, *Lzts1*$^{-/-}$ MEFs showed accelerated mitotic progression, resistance to taxol- and nocodazole-induced M phase arrest, and improper chromosome segregation. Accordingly, Lzts1 deficiency was associated with an increased incidence of both spontaneous and carcinogen-induced cancers in mice.

INTRODUCTION

During the course of cancer development, a normal cell progresses toward malignancy by acquiring a specific series of mutations. Over the past decades, genetic studies have demonstrated that cancer cells accumulate DNA changes that activate oncogenes and inactivate tumor-suppressor genes.

The role of tumor-suppressor genes in neoplasia development is crucial, given that reintroduction of one or more of these genes into cells in which their function is compromised can cause complete reversion to the neoplastic process (Ichikawa et al., 1994). In light of this, it is not surprising that many tumors frequently show loss of heterozygosity (LOH) at specific chromosomal regions encompassing tumor-suppressor genes.

One of these regions is located at chromosome 8p22, which is frequently deleted in breast, lung, gastric, esophageal, prostate, and bladder cancers (Vocke et al., 1996; Wagner et al., 1997; Anbazhagan et al., 1998; El-Naggar

SIGNIFICANCE

Both entry and exit from mitosis are driven by the modulation of Cdk1 activity, which is finely regulated by several protein complexes. Knowing the players that control the activity of Cdk1 in each phase of the cell cycle is of primary importance to fully understand how normal and cancer cells divide. The present data establish *Lzts1* as an important player in ensuring proper Cdk1 activity during M phase. Lzts1 absence in a defined temporal window during M phase results in impaired Cdk1/Cdc25C interaction with a consequent drop in Cdk1 activity. During prophase and metaphase, Lzts1 protein is therefore necessary for maintaining high levels of Cdk1, which, in turn, are required for proper chromosome alignment and segregation.

et al., 1998). On the basis of this correlation and through a positional cloning strategy, we identified a novel putative tumor-suppressor gene (Ishii et al., 1999), *FEZ1/LZTS1* (*LZTS1*), which maps in this region and whose expression is altered in different human malignancies (Ishii et al., 1999; Cabeza-Arvelaiz et al., 2001; Vecchione et al., 2001, 2002; Toyooka et al., 2002; Ono et al., 2003; Nonaka et al., 2005). The reintroduction of *LZTS1* into *Lzts1* null cancer cells suppresses cell growth, and this suppression is probably mediated by *LZTS1*'s interaction with the main mitotic kinase, $p34^{cdc2}$ (hereafter referred to as Cdk1) (Ishii et al., 2001; Vecchione et al., 2002); the majority of cells are seen at the G2/M phases of the cell cycle (Ishii et al., 2001; Vecchione et al., 2002).

Cdk1 activity is finely controlled during the G2/M phases of the cell cycle, and its activity is thought to control both M phase entry and exit (reviewed in Nigg, 2001). There are multiple levels of Cdk1 activity regulation, such as binding with its regulatory subunits (Cyclins A and B), interaction with CKIs, and phosphorylation and dephosphorylation of specific residues (reviewed in Smits and Medema, 2001). Of the several proteins participating in the multifaceted Cdk1 regulation, some have been implicated in tumor progression (Carnero, 2002), directly linking proper M phase progression with cell transformation. However, the involvement of mitotic regulators in cancer is more difficult to prove since profound alterations of M phase are often not compatible with eukaryotic life (Weaver and Cleveland, 2005), a concept in line with the notion that, up to now, any effort to generate Cdk1 knockout (KO) mice has been unsuccessful. Conversely, mild alteration of M phase checkpoints often leads to aneuploidy and cell transformation (Weaver and Cleveland, 2005).

In the present study, we describe the role of the tumor-suppressor gene *Lzts1* in M phase regulation and tumor progression by utilizing *Lzts1* null mice as a model.

RESULTS

Targeted Disruption of the *Lzts1* Gene Creates a Null Allele and Favors Spontaneous Tumor Development

The mouse *Lzts1* gene was disrupted by the insertion a neomycin-resistance cassette that replaced the first *Lzts1* exon (Figure 1A). Chimeric males transmitted the mutated *Lzts1* allele through the germline, as demonstrated by Southern blot analysis of tail DNA (Figure 1B) and by reverse transcriptase-polymerase chain reaction (RT-PCR) of $Lzts1^{+/+}$, $Lzts1^{+/-}$, and $Lzts1^{-/-}$ mouse embryo fibroblasts (MEFs) (Figure 1C). $Lzts1^{-/-}$ mice arose at the expected frequency (data not shown). Western blot analysis of brain lysates from $Lzts1^{+/+}$, $Lzts1^{+/-}$, and $Lzts1^{-/-}$ mice confirmed the reduction or the absence of Lzts1 protein (Figure 1D).

Lzts1 heterozygous intercrosses were bred and observed up to 24 months of age. In animals at the age of 8–24 months (average of 16 months), pathological analysis demonstrated that 0 out of 11 $Lzts1^{+/+}$ mice, 12 out of 20 $Lzts1^{+/-}$ mice, and 9 out of 9 $Lzts1^{-/-}$ mice developed multiple tumors. The neoplasms in $Lzts1^{-/-}$ and $Lzts1^{+/-}$ mice were breast tumors, hepatocellular carcinomas, lymphomas, soft tissue sarcomas, and lung adenomas (Figures 1E–1I), a spectrum suggesting that *Lzts1* absence affects multiple cell types in vivo. Lymphomas, hepatocellular carcinomas, and soft tissue sarcomas developed at a higher penetrance in $Lzts1^{-/-}$ than in $Lzts1^{+/-}$ mice (100%, 75%, and 100% versus 35%, 50%, and 0%, respectively). Penetrance in the other histotype was not significantly different. The age of onset of the tumor was very similar in heterozygous and homozygous mice (19 ± 4.2 months for $Lzts1^{+/-}$ versus 17 ± 4.2 months for $Lzts1^{-/-}$), suggesting that one *Lzts1* allele is not sufficient and does not delay cancer development. To better address this point, we explored whether the second allele was lost in heterozygous mice. PCR experiments coupled with direct sequencing showed that the wild-type (WT) allele was neither lost nor mutated (data not shown).

$Lzts1^{-/-}$ MEFs Progress Normally through the G1, S, and G2 Phases, but Display an Altered M Phase

Our previous studies indicated that *LZTS1* is implicated in the control of cell cycle progression, since its overexpression was able to mainly block the cells in the G2/M phases, and that it probably acts on Cdk1 activity (Ishii et al., 2001; Vecchione et al., 2002). We used MEFs derived from WT or KO embryos to verify whether the mechanisms whereby Lzts1 absence predisposed mice to develop multiple tumors could be linked to its role in the control of cell cycle progression.

In growth-curve experiments, $Lzts1^{-/-}$ MEFs cultured in complete medium grew more slowly over a 6 day period when compared to $Lzts1^{+/+}$ cells (Figure 2A), probably for an increased apoptosis, as demonstrated by flow cytometry analysis (FACS) (Figure 2B) and TUNEL assay (data not shown). In order to assess if the reduced cell growth observed in $Lzts1^{-/-}$ MEFs was also due to an altered progression through the different phases of the cell cycle, we used cell synchronization to study the transition from the G1 to S phases and from the S to G2/M phases. Serum starvation followed by serum stimulation for up to 24 hr indicated that $Lzts1^{+/+}$ and $Lzts1^{-/-}$ MEFs progressed similarly from the G1 to S phases and from the S to G2 phases of the cell cycle (Figure 2C) and demonstrated similar temporal expression and phosphorylation patterns of cell cycle regulatory proteins (Figure 2D). The expression of the licensing factor Geminin, known to be expressed from the S to M phases and degraded at the metaphase-anaphase transition (McGarry and Kirschner, 1998), peaked in both cell lines 23 hr after serum stimulation; however, in cells lacking Lzts1, expression decreased rapidly (Figure 2E).

Using the double-thymidine block coupled with FACS and western blot analyses, we noted a decreased accumulation in the G2/M phases in $Lzts1^{-/-}$ compared to $Lzts1^{+/+}$ cells (Figure 3A). The observed differences in the cell cycle distribution were accompanied by slightly different expression levels of Cyclin B1 (Figure 3B), which, in $Lzts1^{+/+}$ cells, peaked from 4 to 8 hr and decreased promptly 10 hr after release and, in $Lzts1^{-/-}$ cells, rose