ABSTRACT

SMARCAL1 (SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily a-like1) is a distant member of SNF2 family of chromatin remodeling proteins. Mutations in hSMARCAL1 causes autosomal recessive disorder called Schimke Immuno-Osseous Dysplasia (SIOD), a disease characterized by multiple syndromes including chronic-renal failure, T-cell immunodeficiency, cardiovascular disease, hypothyroidism and bone marrow failure. This clearly suggests that hSMARCAL1 might be involved in regulating more than one biological function in a cell. This is not an unusual feature for a protein including that of chromatin remodelers as many proteins participate in more than one cellular function. However, the function of hSMARCAL1 is still not clear and has drawn a serious attention of researchers. Recent studies have reported that the protein has reverse helicase activity and is required for DNA repair during S phase. Another study has shown that deficiency of SMARCAL1 causes cell cycle arrest and developmental abnormalities in a zebrafish model. Further, it has also been shown that SMARCAL1 binds to polytene chromosome and mutation in SMARCAL1 alters such binding and also alters the subcellular localization and enzymatic activity.

The aim of my study is to characterize the function of hSMARCAL1. In order to assign physiological functions to hSMARCAL1, I employed biochemical as well as cell biological techniques. Using protein affinity chromatography followed by mass spectroscopy, β-tubulin was identified as one of the protein partners of hSMARCAL1. As β-tubulin is involved in mitosis, I examined the role of hSMARCAL1 in dividing cells. The protein is excluded from the condensed metaphasic chromatin but remains
associated with spindle fibers near the spindle poles. We speculated that the ATPase activity of hSMARCAL1 might be involved in chromatin condensation and hence spindle pole formation. To study precisely the role of hSMARCAL1 in this event, hSMARCAL1 was downregulated using shRNA. Though, hSMARCAL1 deficiency did not appear to affect chromosome condensation, but was found to affect further events of cell division leading to abnormal mitosis. Large population of cells was found with mitosis related anomalies like cells with multiple microtubule organizing centers, multinucleated cells and increased cell size. We, therefore, postulate that hSMARCAL1 might be playing crucial role in the formation and constitution of spindle poles and fibers or might be involved in regulation of cell cycle checkpoints. Further, the mitotic abnormalities observed due to the downregulation of hSMARCAL1 was also found to affect cell cycle progression. Therefore, our data suggests that downregulation of hSMARCAL1 causes not only mitotic abnormalities, but also alters the normal cell division leading to delayed cell cycle progression and eventually cells decide to undergo programmed cell death. This is consistent with the recent report that knockdown of hSMARCAL1 in zebrafish caused cell cycle arrest at G0/G1 stage, inducing apoptosis.

hSMARCAL1 has been shown to act as an ATP-dependent reverse helicase. Therefore, we analyzed the behaviour of hSMARCAL1 in response to DNA damage, which also directs the cells towards cell death when the damage is beyond repair. Our present data shows that under apoptotic condition hSMARCAL1 co-localizes with apoptotic marker γH2AX at the apoptotic ring as well as at the DNA nicks a hallmark of apoptosis. hSMARCAL1 deficiency showed cells with distinct rounded cell morphology with disassembled DNA, both of which are signs of cells undergoing apoptosis. This
corroborates our data of cell cycle progression getting delayed and ultimately leading to apoptosis as a result of downregulation of hSMARCAL1.

Therefore we speculate that hSMARCAL1 might be involved in multiple cellular functions depending on its subcellular localization in a cell cycle-dependent manner. In S-phase it is reported to be involved in DNA repair and we propose that during mitosis hSMARCAL1 localizes on spindle poles and microtubules thus playing important role in regulating cell cycle. Also, in response to DNA damage it might act as anti-apoptotic factor, as we have observed that the downregulation of hSMARCAL1 leads to DNA-damaged induced cell death.