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Emerging Roles for Human MMS21, NSMCE2, in Development and Disease

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Abstract

The maintenance of chromosome stability during cell division is crucial for inheritance of complete genetic information by progeny cells. Errors in mechanisms safeguarding genomic stability during cell division can result in chromosome aberrations during gametogenesis and development that may manifest as disease phenotypes. Structural maintenance of chromosomes (SMC) protein complexes play important roles in chromosome organization and function and impact a wide variety of chromosomal transactions. The conserved Smc5/6 complex is essential for viability, repair of DNA double strand breaks and recovery of collapsed replication forks and is crucial for chromosome stability. This mini review is focussed on hMMS21/NSMCE2, the human homolog of MMS21/NSE2 that is a non-smc subunit of the Smc5/6 complex having SUMO E3 ligase activity, and aims to provide insights into the role of hMMS21/NSMCE2 in human development and disease associated with deficiency of NSMCE2.

Keywords: MMS21; NSMCE2; SMC; SMC5/6; SUMO-ligase; NSE2; Chromosome abnormalities; Primordial dwarfism; Human developmental defect; Mouse model

Introduction

Structural Maintenance of Chromosomes (SMC) protein complexes are key architectural elements that provide a dynamic scaffolding function to shape chromosomes. SMC complexes are non-histone DNA associated proteins important for higher order chromosome organization [1,2]. SMC proteins usually form V-shaped heterodimers having two ATP-binding head domains attached to coiled regions connected by a hinge region. The SMC heterodimer associates with other subunits that are integral to the function of the SMC complex. Several chromosomal structural attributes such as condensation and sister chromatid cohesion are regulated by SMC complexes such as condensin and cohesin [1,3]. In addition, these SMC-complexes are involved in numerous other chromosomal transactions such as DNA double strand break repair, replication, regulation of gene expression, etc. [1,4-7]. Deficiency in the genes encoding these complexes, particularly cohesin and its loading factors, is associated with severe developmental defects in humans that are being investigated extensively [8]. A third as yet un-named SMC complex, the Smc5/6 complex [9], is also a chromatin associated, essential complex, whose Smc subunits are more closely related to bacterial Smc-related proteins compared to Smc1 or 3 or Smc2 or 4, that constitute the Smc heterodimers in cohesin and condensin, respectively.

The enigmatic Smc5/6 complex is evolutionarily conserved from bacteria to humans. While its function remains elusive, there is clear evidence of its involvement in multiple chromosomal processes. The Smc5/6 complex plays an important role in mitotic proliferation as well as meiosis, and in maintenance of chromosome stability [10-12]. It is involved in rescue of collapsed replication forks [13,14], progression of replication forks [15,16] DNA double strand break repair and resolution of recombination intermediates [17,18]. The Smc5/6 complex in eukaryotic cells such as yeast has eight subunits that include Smc5, Smc6, and six non-Smc subunits (Non Smc Elements or Nse1-6), all of which are essential for viability. Intriguingly, in addition to the ATPase activity of the Smc5/6 heterodimer, the Smc5/6 complex possesses two additional enzymatic activities. The Nse1 subunit has a RING-domain and is a ubiquitin ligase that forms a subcomplex with Nse3, a MAGE (Melanoma associated antigen) domain containing protein [19]. The Nse2 subunit, originally named as Mms21, is a SUMO-E3 ligase that sumoylates a variety of chromosomal proteins [9,20].

Mms21/Nse2 in yeast cells (hereafter referred to as Mms21) is essential for cell viability and has an SPL-RING (Siz/PIAS Like - Really Interesting New Gene) domain near its C-terminal end. Mms21 is required for resistance to genotoxic stress; it was first discovered in a screen for MMS (Methyl methane sulfonate)-sensitive mutants [20]. SUMO-ligase deficient *mms21* mutants in budding yeast, having mutations in the SPL-RING domain, are viable, but are sensitive to DNA damage inducing agents such as MMS, HU (hydroxyurea) and ultra-violet (UV) radiation[9,10]. While the SUMO-ligase deficient cells are viable, in budding yeast, they are slow growing and display enhanced chromosome loss and breakage coupled with dependence on active DNA-damage signalling mediated by Mec1, the yeast homolog of the phosphatidyl inositol 3-kinase ATM (Ataxia telangiectasia mutated) protein [10]. Mms21 is required for resolution of X-shaped DNA intermediates at MMS-damaged replication forks [18] and relocation of DNA double strand breaks to the nuclear pore for repair [21]. Human MMS21/NSE2 (hMMS21 or NSMCE2) is relatively less characterized; it is also a SUMO ligase that sumoylates hSMC6 and the DNA repair protein TRAX and is required for DNA repair and prevention of DNA-damage induced apoptosis [22]. In telomerase deficient cancer cells that exhibit alternative lengthening of telomeres (ALT cells) by telomere homologous recombination, hMMS21 sumoylates telomere binding proteins TRF1 and TRF2 and facilitates recruitment of telomeres to PML bodies known as APBs [23].

A novel genetic syndrome associated with mutation in human *NSMCE2* was reported by Payne et al. [24] recently. Two female patients displaying primordial dwarfism were identified, who

harboured compound heterozygous frame-shift mutations in both alleles of the *NSMCE2* gene. The S116Lfs*18 mutation deleted a large part of the C-terminal region including the entire SUMO ligase RING domain replaced by 18 unstructured amino acids, while the A234Efs*4 mutation substituted the last 14 amino acid residues with 3 residues. The truncated mutant protein corresponding to S116Lfs*18 was not readily detectable in the patient's cells whereas the A234Efs*4 mutant protein was less stable and expressed at a lower level in patient derived LCL (Epstein Barr virus immortalized lymphoblastoid cell lines) and dermal fibroblasts. The S116Lfs*18 mutant protein was auto-sumoylation defective while the auto-sumoylation of the A234Efs*4 mutant protein was not significantly different from the wild-type NSMCE2 protein [24]. However, the patient derived LCLs showed mild reduction of SMC5 and SMC6 proteins in whole cell extracts and chromatin fractions indicating some instability of the Smc5/6 complex in these cells. Hence, hypomorphism in NSMCE2 is also accompanied by mild reduction in SMC5 and SMC6 functions that may also contribute to cellular and developmental defects in the patients.

The NSMCE2 hypomorphic patients displayed multiple developmental defects most notably primordial dwarfism, facial dysmorphism, primary gonadal failure and insulin resistant diabetes [24]. Numerous cellular defects were also observed in patient dermal fibroblasts and LCLs. The frequencies of micronuclei (MN) that may form from acentric broken chromosome fragments, and of nucleoplasmic bridges (NPB) that represent unresolved chromosome strands connecting two nuclei of a binucleate cell prior to cytokinesis, are indicative of chromosome instability, and were enhanced in patient cells especially following treatment with HU that induces replication fork stalling and collapse [24]. In addition, patient derived LCLs treated with low concentrations of HU showed impairment in recovery of replication relative to wild-type. Both LCLs and fibroblasts from patients showed defect in formation of BLM foci in response to HU and ionizing radiation compared to wild-type cells. Following UV treatment that also induces replication fork stalling, there was enhanced formation of UV-induced sister chromatid exchanges (SCE) in patient derived LCLs. These findings [24] indicate that chromosome instability events are more prevalent in NSMCE2 hypomorphic cells. It is possible that the associated replication delay or cell cycle arrest may contribute to the growth impairment observed in patients. Furthermore, primary gonadal failure, in particular ovarian failure in the two identified patients having the *NSMCE2* mutations, may be a consequence of the role of NSMCE2 in meiosis or mitotic division of germ cells during development, by analogy with the known function of Mms21/Nse2 and Smc5/6 in meiosis and mitotic divisions in model organisms such as yeasts [10,11,25,26] and human cell lines [27].

Further insight into the role of NSMCE2/hMMS21 in human cells that may shed some light on disease associated growth defects comes from additional studies on cellular functions of NSMCE2 in cell lines of different lineages. Depletion of hMMS21 in MCF-7 breast cancer cells results in slow G1-S progression when serum starved hMMS21 depleted cells are supplemented with serum containing medium [28]. This correlates with down regulation of E2F1 and Cyclin E/CDK2 that are important for the G1-S transition, in hMMS21 depleted cells. Such effects may contribute to additional slowing down of cell cycle progression resulting in reduced growth.

Recent studies using murine C2C12 myoblasts have demonstrated that sumoylation also plays a key role in myogenic differentiation; inhibition of global sumoylation by ginkgolic acid or by si-RNA mediated knock-down of Ubc9, the SUMO conjugating E2 enzyme, results in repression of expression of a subset of myogenic differentiation markers and delay in appearance of morphological hallmarks of differentiation [29,30]. Knock down of *Nse2* gene expression in C2C12 cells also inhibits myogenic differentiation [30]. Murine Mms21/Nse2 binds skNAC, a skeletal and heart muscle-specific variant of α subunit of nascent polypeptide associated complex that also binds m-Bop/Smyd1, a sumoylated multi-functional protein that regulates myogenesis. Depletion of Nse2 in C2C12 cells blocks nuclear to cytoplasmic translocation of skNAC-Smyd1 complex during myogenic differentiation causing its retention in PML (Promyelocytic leukemia)-like nuclear bodies and results in defective sarcomerogenesis [30]. These findings indicate a role for murine Nse2 in facilitating myogenic differentiation and if this function is conserved in humans, it may provide a clue to the cause of dwarfism and deficit of muscle mass observed in NSMCE2 hypomorphic patients.

Another recent study using the mouse model system [31] investigated the role of murine homolog of NSMCE2 *in vivo*, in early development and in adult mice. A mutation *Nsmce2*^{GT} that removes the coding sequence beyond the 88th amino acid resulted in termination of embryonic development at approximately 3-3.5 dpc. Mutant *Nsmce2*$^{GT/GT}$ morulae displayed nuclei of irregular size and shapes, condensed chromosomes and some cells of abnormal size, indicative of chromosome segregation defects that may cause early embryonic lethality. Surprisingly, mutation of the SUMO-ligase domain by substitution of two conserved Cysteine and Histidine residues that reduce auto-sumoylation activity *in vitro*, had no effect on embryonic development. However, it could not be established that the sumoylation of NSMCE2 targets was indeed reduced in the mutant mice *in vivo*; hence the role of NSMCE2 mediated sumoylation in mouse development remains unclear from this study. Interestingly, Payne et al. [24] showed that depletion of the zebrafish NSMCE2 ortholog causes dwarfism that can be rescued by wild-type NSMCE2 but not the SUMO-ligase defective variant indicating that NSMCE2 SUMO ligase activity is indeed important for development in a vertebrate model. In mice *Nsmce2* haploinsufficiency in *Nsmce2*$^{GT/+}$ cells, that show nearly 50% reduction in NSMCE2 protein levels, is associated with increased sister chromatid exchanges (SCEs), micronuclei, polynucleated cells and enhanced incidence of tumors demonstrating that NSMCE2 is a haplo insufficient tumor suppressor in mice [31]. Interestingly, deletion of *Nsmce2* in adult *Nsmce2*$^{lox/lox}$ mice carrying the UQ.CreERT2 transgene, by exposure to 4-OHT (4-hydroxytamoxifen), resulted in a progressive generalized progeroid syndrome (displaying phenotypes reminiscent of accelerated aging) accompanied by several pathological hallmarks of Bloom's syndrome such as reduced fat, altered pigmentation and anemia [31]. However, NSMCE2 operates independently of BLM in these cells [31].

MMS21 has multiple roles in cells arising from its ability to sumoylate a wide variety of protein targets involved in diverse cellular processes. While its roles in chromosome stability are well appreciated and arise from its ability to modify numerous chromosomal proteins involved in chromosome organization, segregation and repair [9,10,12], new roles in nuclear-cytoplasmic transport and metabolism are being identified. Mms21 in budding yeast, sumoylates the nuclear import receptor Kap114, that is required for import of transcription factors and other cargos across the nuclear pore [32]. Sumoylation defective Kap114 accumulates in the nucleus and may be defective in unloading cargos ultimately resulting in impairment of import of Kap114 cargos. Another target of Mms21 is Snf1kinase, a regulator of carbon metabolism [33]. Mms21 mediated sumoylation of Snf1, in

response to glucose and DNA damage, inhibits its activity and targets it for degradation via the Slx5-Slx8 ubiquitin ligase [33,34].

In conclusion, mammalian MMS21/NSMCE2 is critical for normal development, chromosome stability and tumour suppression *in vivo*. Identification of the full spectrum of sumoylation targets of hMMS21/NSMCE2 in human cells and determination of the functional relevance of NSMCE2 mediated sumoylation in different cell types and developmental lineages may reveal more insights into understanding all the defective phenotypes associated with NSMCE2 dysfunction in humans. Understanding the SUMO-ligase independent function of NSMCE2 is also critical in fully understanding disease phenotypes associated with NSMCE2 hypomorphism.

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