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Brg1: A range of functions during eye development

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1. Structure

Chromatin structure controls all processes related to the storage and expression of genetic information. “Chromatin remodeling” refers to those processes that alter local and/or global chromatin organization. This process is executed by chromatin remodeling enzymes, many of which form multiprotein complexes with “core” catalytical subunits. The SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (Smarca4, UniProtKB/Swiss-Prot accession P51532, EC=3.6.4.-), also known as Brahma-related gene 1 (Brg1), serves as a catalytical subunit of the SWI/SNF (Switch/Sucrose Non-fermentable) ATP-dependent chromatin remodeling complexes. SWI/SNF complexes contain 11 additional subunits termed Brg1-associated proteins (BAFs). Brg1, and its sibling protein Brm, belong to the SNF2/RAD54 helicase family of enzymes that possess a 47 kDa DEAD/H helicase domain comprised of DEXDc and HELICc regions (Fig. 1A). In addition, an N-terminal proline-rich region mediates a range of specific protein-protein interactions. An internal helicase/SANT-associated (HSA) domain at its N-terminus facilitates the interaction between β -actin and actin-related BAFs. The C-terminal Brg1 bromodomain, a four-helix bundle motif, binds to acetylated histones, and is thought to play a role in the recruitment of SWI/SNF complexes to specific regions of the chromatin. Two remaining structural motifs include an LxCxE Rb binding motif, also known as E7 homology region, and a short KR region, enriched in lysine and arginine residues. The KR region, also known as AT-hook, participates in DNA binding and may function as a nuclear localization signal (Fig. 1A).

2. Function

The molecular function of SWI/SNF complexes is to non-covalently alter the DNA-histone interactions by using energy from ATP hydrolysis. Single molecule experiments with purified nucleosomes suggested directional DNA translocations catalyzed by Brg1 that moves nucleosomes along the DNA and can lead to displacement of nucleosomes and/or generation of nucleosome-free regions (Yoo and Crabtree, 2009). In terms of gene expression, the SWI/SNF complexes can serve as both activators and repressors. The same

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promoter can be either activated or repressed by Brg1-containing complexes depending on the cellular context. This switch can be mediated by ligand-binding to nuclear receptors or through recruitment of histone acetyltransferases (e.g. p300 and CBP) or histone deacetylases (HDAC) for activation and repression, respectively. During neurogenesis, the neural progenitor-specific BAF53a is being replaced by post-mitotic BAF53b through the repressor-element-1-silencing transcription factor (REST)-mediated repression of microRNAs miR-9* and miR-124 (Yoo and Crabtree, 2009). Similarly, another subunit BAF60c, which is expressed in neuronal/retinal progenitors, is required for their proliferation via the Notch pathway, and its expression is lost during neural differentiation (Lamba et al., 2008).

Additional mechanisms of transcriptional regulation by Brg1 (SWI/SNF) were proposed. SWI/SNF complexes mediate eviction of the polycomb (PcG) protein complexes PCR1 and PCR2 at silencers, followed by recruitment of MLL1 histone methyltransferase and departure of DNA methyltransferase DNMT3b. This alters local chromatin structure to facilitate transcriptional activation. Another scenario is based on the complex formation between SWI/SNF and neuron-restrictive silencer factor (NRSF), its co-repressors SIN3A and CoREST, and regulation of the amount of linker histone H1 at the silencer regions. Accumulation of H1 results in eviction of the repressor from the silencer. The Brm-containing SWI/SNF complexes bind to the methyl-CpG binding protein MeCP2, serving as a universal transcriptional repressor.

Studies of Brg1 showed tissue-specific roles of this enzyme in neuronal, retinal and lens development among other tissues. A somatic mutation of Brg1 generates the zebrafish *young (yng)* mutants responsible for multiple lens and retinal defects. Studies of *Brg1/yng* during retinal differentiation suggest that Brg1 might act downstream of Shh and Ath5 and participate in the induction of cell cycle exit and differentiation (Leung et al., 2008). Mouse studies also found that Brg1 is important for neural stem cell and progenitor cell maintenance and is involved in the regulation of Shh and Notch signaling components (Leung et al., 2008; Yoo and Crabtree, 2009).

In lens, recent studies established cell-autonomous roles of Brg1 in lens fiber cell terminal differentiation and denucleation (He et al., 2010). In transgenic lenses, ectopic expression of dominant negative Brg1 in postmitotic lens fibers disrupted their terminal differentiation (Fig. 1B). Similarly, a range of lens differentiation defects following conditional inactivation of Brg1 in the surface ectoderm that give rise to the lens and corneal epithelium, were observed (Fig. 1B). In both models, lens fiber cell nuclei were not degraded and the organelle-free zone (OFZ) was absent. RNA expression profiling identified reduced expression of DNase II β in both systems, raising the possibility that expression of DNase II β , an essential enzyme which degrades lens chromatin, is directly regulated by Brg1 (He et al., 2010). It has also been shown that mammalian SWI/SNF complexes participate in DNA double-strand break (DSB) repair and DNA replication. Inhibition of Brg1 leads to deficiency in DNA DSB repair, and consequently, increased sensitivity in DNA damage. Thus, lens fiber cell denucleation defects can be caused by multiple and mutually not exclusive molecular mechanisms.

3. Disease Involvement

To date, the exact role of Brg1 in eye diseases has not been established; however, Brg1 mutations have been linked to cancer. One of the many examples is the rhabdoid tumor predisposition syndrome-2 (RTPS2), which is caused by the loss of heterozygosity at the *BRG1* gene locus (Fig. 1A). Since Brg1 was shown to activate the transcription of cyclin-dependent kinase inhibitors and Brg1 forms a complex with pRb, it is reasonable to

hypothesize that *BRG1* mutations and its deregulation might play significant roles in retinoblastoma and other ocular tumors such as melanomas.

4. Future Studies

The role of Brg1 in mammalian retinal development and differentiation of individual retinal cell types requires further experimentation using cell type specific conditional knockouts in mouse. Additional insight into this process will be gathered via studies of tissue-specific regulatory BAFs such as BAF53a, BAF53b, and BAF60c. Interesting mechanistic data should be revealed from studies of DNA-binding transcription factors known to associate with Brg1 (e.g. Hsf4 and Pax6), examination of pRb/Brg1 complexes mediated by the LxCxE pRB-binding motif (Fig. 1A), and from unbiased proteomic studies of Brg1-associated proteins. In lens, the novel role of Brg1 to control the process of lens fiber cell denucleation cannot be explained by down-regulation of DNase II β only (He et al., 2010). An intriguing possibility is that DNA repair machinery is activated prior to the denucleation process with active participation of chromatin remodeling catalyzed by Brg1. It is also possible that one or more components of the DNA repair system performs dual roles, earlier to facilitate DNA repair in postmitotic lens fibers (E12.5-E15.5) followed by organized degradation of the chromatin. Thus, it will be important to determine chromosomal distribution of Brg1 together with the components of DNA repair machinery (e.g. phosphorylated H2AX and DNA repair protein Nbs1) and associated core histone modifications including H3 K36me2 and H4 K20me2 using ChIP-seq.

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Fig. 1.

(A) Human *BRG1* gene spans 101,362 bps on chromosome 19 (region p13.2) and has at least 9 different splice variants. The BRG1 protein (1,647 amino acid, 184,646 kDa) is comprised of multiple functional subdomains: ATPase subdomain [DEXDc (orange) and HELICc (green) homology subdomains, amino acid residues 766 to 1,246], bromodomain (yellow, amino acid residues 1,477 to 1,547), E7 homology (pRb binding motif, blue), helicase/SANT-associated subdomain (HSA, purple), KR (amber), and proline-rich (AT-hook, DNA-binding, pink) subdomains. (B) Ocular defects in transgenic mouse overexpressing catalytically inactive Brg1 (dnBrg1) in the lens, and in Brg1 lens conditional knockout (Brig1 cKO). All stages are newborn embryos (P1). Cornea, C; lens epithelium, LE; neural retina, NR; organellae free zone, OFZ; lens transitional zone, T; wild type, wt. Scale bar, 100 μ m.