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Exploring the developmental mechanisms underlying Wolf-Hirschhorn Syndrome: Evidence for defects in neural crest cell migration

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ABSTRACT

Wolf-Hirschhorn Syndrome (WHS) is a neurodevelopmental disorder characterized by mental retardation, craniofacial malformation, and defects in skeletal and heart development. The syndrome is associated with irregularities on the short arm of chromosome 4, including deletions of varying sizes and microduplications. Many of these genotypic aberrations in humans have been correlated with the classic WHS phenotype, and animal models have provided a context for mapping these genetic irregularities to specific phenotypes; however, there remains a significant knowledge gap concerning the cell biological mechanisms underlying these phenotypes. This review summarizes literature that has made recent contributions to this topic, drawing from the vast body of knowledge detailing the genetic particularities of the disorder and the more limited pool of information on its cell biology. Finally, we propose a novel characterization for WHS as a pathophysiology owing in part to defects in neural crest cell motility and migration during development.

1. Introduction

Wolf-Hirschhorn Syndrome (WHS) is a developmental disorder characterized by intellectual disability, craniofacial abnormalities, heart defects, skeletal defects, urogenital defects, and seizures (Battaglia et al., 2015; Cooper and Hirschhorn, 1961; Wolf et al., 1965). The most obvious and common clinical marker of WHS is the “Greek warrior helmet” appearance, caused by an abnormally wide nasal bridge attaching the nose to the forehead. WHS patients also exhibit a high forehead, drastic eyebrow arches, widely spaced eyes, a short philtrum, and micrognathia (undersized jaw). The vast majority of patients are microcephalic and have ears marked by abnormal positioning on the head and underdeveloped cartilage. Defects of the eye and optic nerve also occur in approximately 40% of patients. Many facial phenotypes can be classified as midline defects, including the common cleft palate. An overarching growth delay is also notable in WHS patients; the onset is prenatal in most and continues to manifest throughout early development, as patients’ stature is short and weight gain is slow (Battaglia et al., 2015). As the pathways responsible for brain development and craniofacial morphogenesis are linked (Brault et al., 2001; Marcucio et al., 2005), it is not surprising that a majority of WHS patients also exhibit mental retardation (Bergemann et al., 2005).

Each patient presents with a unique combination of WHS characteristics, with varying severity. Such clinical variability is the first reason that it has proven difficult to conduct research into the disorder's underlying pathology. This review will focus on a second reason: WHS manifests at the genomic, epigenomic, and cell biological levels, and there is a lack of mechanistic knowledge regarding the exact effectors downstream of certain genetic mutations. Consequently, the field still lacks insights into appropriate initial questions to jumpstart investigations into the cell biological functions of WHS-related proteins. However, several players in the processes underlying the disorder have previously been described —for the most part, these are proteins which have established roles in routine epigenetic modification, normal cellular metabolism, or in signaling pathways integral to proper development. From a few early studies, it has become clear that some of these epigenetic modifications and signaling events are connected to cell motility-related processes. Furthermore, the characteristic facial (and cardiac) phenotypes of WHS call to mind the neural crest, as most vertebrate facial features are derived from or influenced by the cranial subset of this multipotent stem cell population (Bronner and LeDouarin, 2012). Neural crest cells are born along the embryonic neural tube, and migrate long distances to reach their destinations, where they differentiate and contribute to structures such as peripheral nerves, jaw structures, facial cartilage, elements of the heart, and pigmented epidermal tissue (Bronner and LeDouarin, 2012). Considering both the morphological data detailing the WHS phenotype and recent cell biological studies exploring WHS candidate proteins, neural crest cell motility and migration are a promising avenue for investigation into WHS at the cell biological level.

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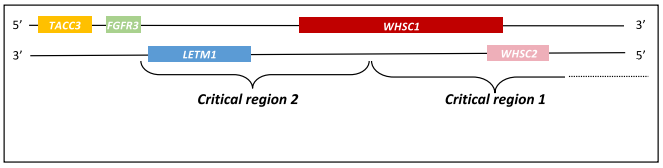

Fig. 1. Chromosome 4p16.3 and WHS candidate genes. Genes are depicted in their order from left to right, telomeric to centromeric orientation. Sizes are proportional to the length of the gene in kilobases. Plus or minus strand orientation is also represented. Brackets represent the lengths of the two WHS critical regions. As dotted line indicates, figure scaling is such that critical region one is not represented in its entirety on the page.

Following Hirschhorn's initial characterization of a clinical phenotype in 1961, the major catalyst for research into WHS has been the definition of a critical genomic region ("critical region 1"), consisting of 165 kilobases on the short arm of chromosome four (the 4p region), which is deleted in traditional cases of the disorder (Wright et al., 1997; Fig. 1). The only characterized candidate genes that fall within this region are Wolf-Hirschhorn candidate 1 (*WHSC1*), referred to elsewhere as a multiple myeloma SET (MMSET) or nuclear receptor SET domain (NSD) protein, and *WHSC2*, which is also known as a negative elongation factor (NELF) (Wright et al., 1999). Despite lying entirely within the WHS critical region, *WHSC2* has not emerged as one of the primary contributors to the WHS phenotype. While this may be due to a deficit in *WHSC2*-based studies, it also results from the identification of several patients with deletions telomeric to the critical region. As *WHSC2* is left unaffected in these cases, they have called into question the gene's clinical relevance (Bergemann et al., 2005; Cyr et al., 2011; Engbers et al., 2009; Zollino et al., 2014).

Deletions in the 4p region in most WHS patients encompass areas extending beyond the critical region, thus affecting several flanking genes including a transforming acidic coiled-coil gene (*TACC3),* a sequence encoding a fibroblast growth factor receptor product *(FGFR3)*, and the gene for a leucine zipper and EF-hand containing transmembrane protein *(LETM1),* as listed in their sequential order with *TACC3* most telomeric (Zollino et al., 2003). More recent genomic characterizations have revealed synteny with genes found on chromosome 8p11.2 (Stec et al., 2001). It is clear that the *FGFR-LETM-WHSC1* ordering is ancient, as it is conserved from fish to humans. Moreover, there are speculations as to whether this conserved region is larger, including *TACC* and *NELF/WHSC2,* and whether this particular grouping indicates some functional relationship (Stec et al., 2001). This review will explore the latter possibility by focusing in on the WHSC1 protein encoded on chromosome 4p16.3 (where WHS mutations typically occur), as well as on FGFR3, LETM1, and TACC3, as these are presently the most promising candidates that may demonstrate a novel functional relationship based on neural crest cell motility in WHS (Table 1). Clinical variation in 4p deletion size has highlighted other genes (*SLBP, CTBP1, CPLX1, PIGG, FGFRL1*) as potential WHS contributors (Battaglia et al., 2015); however, a smaller group are discussed in this review to best articulate potential cell biological intersections between specific candidate proteins with putative roles in neural crest cell migration. Importantly, hemizygous deletions in the 4p region have proven sufficient for the onset of traditional WHS (Battaglia et al., 2015). In such a conserved and vital region, it is worthwhile to consider each candidate protein alongside its genomic neighbors; this inclusive approach may illuminate ways in which aberrant regulation of several WHS candidate proteins may converge to impact cell migration events which are crucial for early morphogenesis and neuronal wiring.

2. WHSC1

Of all the candidate genes, *WHSC1* is most often found to be partially or fully monosomic in a majority of clinical cases; however, its absence does not account for the full range of phenotypes associated with the disorder. *WHSC1* is a 90 kb gene, two-thirds of which extend into the telomeric end of the 165 kb Wolf-Hirschhorn Syndrome Critical Region. It contains 25 exons and is subject to complex alternative splicing (Stec et al., 1998). Northern blot analysis of expression patterns in human and mouse embryonic and fetal tissues are highly variable and suggest that many different tissue-specific transcripts exist, with 9 kb and 6 kb transcripts consistently appearing across tissue types. Between exons 4 (where the translational start site is located) and 25, there is a 4095 base pair open reading frame (ORF) from which the WHSC1-encoding mRNA is transcribed (Stec et al., 1998). *In situ* hybridization in mouse revealed a specific pattern of *WHSC1* expression in the developing nervous system at day 10.5, and expression in brain, ganglia, neural tube, jaw, face, intestinal and lung epithelium, liver, adrenal, and urogenital region at day 13.5. Such an expression pattern is significant given that many of these—notably the brain, jaw, face, and urogenital structures—are affected in the classical WHS phenotype (Stec et al., 1998), and given that the formation of structures of the face and jaw, as well as certain glial cell populations in the brain, depend on neural crest cell migration (Bronner and LeDouarin, 2012). However, WHSC1 function has not yet been explicitly linked to neural crest cell migration.

Table 1
WHS candidate genes. For each gene, the potentially WHS-related functions of its protein product are summarized, and relevant references detailing genetic, biochemical, and clinical data related to the gene are listed.

Gene	Function	References
TACC3	Transforming acidic coiled-coil protein: microtubule plus-end tracker, co-factor of the hypoxia inducible factor complex, regulator of epithelial-to-mesenchymal transition and cell migratory behaviors.	Gergely et al., 2000; Groisman et al., 2000; Peset and Vernos, 2008; Simon and Bergemann, 2008; Cyr et al., 2011; Zollino et al., 2014; Nwagbara et al., 2014.
FGFR3	Fibroblast growth factor receptor: involved in neural crest cell chemotaxis, regulator of cell proliferation, cell survival-promoting roles in neural development.	Colvin et al., 1996; Deng et al., 1996; Saarimäki-Vire et al., 2007; Puligilla et al., 2007; Simon and Bergemann, 2008; Katoh and Nakagama, 2014; Sato et al., 2011; Eswarakumar et al., 2005.
LETM1	Leucine-zipper and EF-hand-containing transmembrane protein: involved in mitochondrial bioenergetics, putative roles in regulating the cell cycle and cellular calcium homeostasis.	Endele et al., 1999; Zollino et al., 2003; Nowikovsky et al., 2004; Schlickum et al., 2004; Jiang et al., 2013; Doonan et al., 2014a, 2014b.
WHSC1	Histone H3 lysine methyltransferase; epigenetically influences TWIST transcription, interacts with beta-catenin.	Stec et al., 1998; Toyokawa et al., 2011; Ezponda et al., 2013.
WHSC2	mRNA processing and cell cycle regulation.	Wright et al., 1999.

2.1. WHSC1 as a histone modifier during development

Although there is a reigning generalization in the field that abnormal *WHSC1* dosage is responsible for many of the defining phenotypes of WHS (Bergemann et al., 2005), the question of how it causes such phenotypes remains unanswered. The protein product of this gene is also called MMSET or NSD2, as it is characterized by several domains with known importance to development, including an HMG box, a PHD-type zinc finger domain, and a SET domain, all involved in regulation of transcription during development (Stec et al., 1998). The SET domain possesses methyltransferase capabilities and is common to all but one member of the histone lysine methyltransferase family (Marango et al., 2008; Völkel and Angrand, 2007). Experiments using recombinant NSD proteins have shown that these histone lysine methyltransferases can specifically dimethylate lysine 36 of histone H3