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Characterization of *Sgo1* expression in developing and adult mouse

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ABSTRACT

SGO1 has been characterized in its function in correct cell division and its role in centrosome cohesion in the nucleus. However, its organ-specific maturation-related expression pattern in vivo remains largely uncharacterized. Here, we show clear *SGO1* expression in post-developmental neuronal cells and cytoplasmic localisation in nucleated cells with a transgenic mice model and immunohistochemistry of wild type mice. We demonstrate extranuclear expression of *Sgo1* in the developing heart and gut, which have been shown to be dysregulated in humans with homozygous *SGO1* mutation. Additionally, we show *Sgo1* expression in select population of retinal cells in developing and post-developmental retina. Our expression analysis strongly suggests that the function of SGO1 goes beyond its well characterized role in cell division.

1. Introduction

At the prophase of animal cell division, sister chromatids are held together with centromeric protein complex called cohesin until the chromatids are separated by the spindle apparatus at metaphase. Shugoshin-1 (SGO1), is a member of the cohesin protein complex and prevents the removal of this cohesin ring until the end of metaphase. Depletion of SGO1 in Hela cells causes premature loss of centromeric cohesin, precocious segregation of sister chromatids, and mitotic arrest (McGuinness et al., 2005). Mice haploinsufficient for *Sgo1* are fertile and viable, but develop a propensity towards colon tumors due to chromosomal instability (Yamada et al., 2012). Similar findings have been made in humans: somatic mutations of *SGO1* have been identified in colon cancer and downregulation of *SGO1* has been associated with chromosomal instability (Iwaizumi et al., 2009; Kahyo et al., 2011) demonstrating the significant role of SGO1 in cell division.

Interestingly, a rare non-synonymous mutation of *SGO1* in humans has been shown to cause a generalized pacemaking disorder specifically affecting heart and gut rhythm termed Chronic Atrial and Intestinal Dysrhythmia (CAID) syndrome. Patients affected with CAID syndrome present with clinically significant sinoatrial node dysfunction ("sick sinus syndrome") and chronic intestinal pseudo obstruction at an age between 5 and 40. More precisely, CAID syndrome is caused by a recessive missense mutation of a highly conserved amino-terminal lysine residue to glutamic acid (K23E). Knockdown of *sgo1* in zebrafish causes bradycardia, a characteristic of a sick sinus syndrome seen in CAID (Chetaille et al., 2014).

The organ-specificity and the untimely early onset of electrophysiological symptoms of CAID syndrome patients prompted us to characterize *Sgo1* expression pattern during mouse development with a focus on the affected organs. With a *Sgo1*-lacZ-knock-in mouse model and immunohistochemistry of wild type mice, we here show noteworthy *Sgo1* expression in heart, gut, eye and other central nervous system tissue during development and post-development. The study furthermore demonstrates *Sgo1* localisation in cytoplasmic region in nucleated cells in select organs.

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2. Results

2.1. Sgo1 expression is pervasive in early development and most robust in the developing heart and eye

Sgo1$^{+/LacZ}$ mice (het) were generated using the tm1a gene trap vector. In *Sgo1*$^{+/LacZ}$ mice, the tm1a was inserted between exon4 and 5 of *Sgo1* (Fig. 1). Thus, the expression of lacZ is under the control of the endogenous locus, allowing assessment of *Sgo1* expression using X-gal staining. This mouse model also contains multicistronic elements flanking the LacZ sequence which results in separate translation products of truncated N-terminal SGO1 and β-galactosidase. *Sgo1* $^{LacZ/LacZ}$ mice were embryonic lethal with no homozygous knock-in mice found as early as E9.5 (data not shown).

In order to obtain an overview of *Sgo1* expression, we performed whole-mount X-gal staining of E9.5, E10.5, E12.5 and E15.5 *Sgo1*$^{+/LacZ}$ embryos. During this developmental time frame, we found a ubiquitous, low to medium level of expression throughout the embryo (Fig. 2A, E, 3A, 3E). Wild type embryos were X-gal stained as a negative control under the same conditions and did not show any background signal (Fig. 2D, H, 3D, 3H). We defined the low level pervasive expression intensity of *Sgo1* as the baseline, then focused on organs and tissues that displayed a localised and noticeably higher expression than this baseline at the whole embryo level. X-gal staining at the luminal border of the intestine described in Figs. 6 and 7 were not visually prominent from the gross observation.

At E9.5, we observed low to medium intensity of pervasive expression throughout the embryo with higher expression in the neuroectoderm (Fig. 2A). The heart at this stage did not show higher expression than the rest of the embryo (Fig. 2B). E10.5 showed markedly higher expression from the baseline in the forebrain, heart, and eye (Fig. 2E, F, G respectively). Branchial arches and limb buds also showed noticeable expression (Fig. 2E and F). E12.5 showed high level of expression in the forebrain, heart and eye (Fig. 3A, B, C). At E15.5 we observed a high level of expression in the developing skin (Fig. 3E). At E15.5 and beyond, despite overnight permeabilization with detergent, considerably larger thickness of the embryo and further development of the epidermis did not allow complete penetration of X-gal within 36 hours of incubation. We therefore isolated each organ which had shown high expression in the earlier stages and performed the X-Gal assay directly on the isolated organs. E15.5 mice showed a high level of expression throughout the entire heart (Fig. 3F). E15.5 eyes revealed a pervasive, low level of expression (Fig. 3G, DiB Fig. 1E). A relatively thick retina with low-level staining of the whole eye accounted for an impression of a higher level of staining at a gross level.

2.2. Sgo1 is strongly expressed in the developing heart and displays extranuclear localisation

In mouse hearts, *Sgo1* was expressed in all stages studied. In E9.5 hearts, cardiac X-gal signals showed no marked difference from the baseline signal level of the embryo. Furthermore, the X-gal signal within the E9.5 heart was distributed evenly. Immunostaining with anti-SGO1 antibody also displayed a constant level of signal throughout the heart (Fig. 4A, E). Whole X-gal stained embryo sections at E9.5 and E10.5 highly resembled its' immunostained counterpart (DiB Fig. 1A, B, C, D). At E10.5, the X-gal signal in the heart was notably stronger than the rest of the embryo, in agreement with the pattern observed by immunostaining. Within the E10.5 heart, the X-gal signal was strongest at the left atrial appendage (Fig. 4B). The ventricles showed high and even distribution of the X-gal signal. Immunostaining did not show a higher signal specifically at the left atrial appendage; however, it showed robust DAB signal throughout the entire common atrial wall (Fig. 4F, DiB Fig. 1B'). Hearts of E12.5 embryos also displayed comparable X-gal signals to E10.5, with somewhat stronger signal intensity in the rostrolateral region which was not replicated in the immunostain counterpart of this region. Interestingly, the endocardial cushion had considerably less X-gal signal; immunostaining reproduced this pattern (Fig. 4C, G). X-gal staining in the endocardial cushion was also low in E15.5 hearts, shown in the transverse section near the atrio-ventricular junction. This result was also replicable in immunostaining. Overall, X-gal signal in E15.5 hearts was stronger and more widespread in comparison to the earlier stages, expressed in the entirety of the ventricular and atrial wall as well as the aorta and pulmonary trunk (Fig. 4D, H). DAB signal within the cardiac chambers is caused by blood cells which react with the DAB reagent.

Next we examined the intracellular localisation pattern of SGO1. In principle, the X-gal knock-in mice produce SGO1 protein that is separate from the β-galactosidase; thus, immunostaining was also used as a method to study intracellular localisation. Interestingly, DAB signal largely localised outside the methyl green stained nuclei in the cytoplasmic region (Fig. 5A–D). Testis spermatogonium is highly mitotic (Cai et al., 2011). SGO1 localisation pattern in mitotic cells, such as spermatogonium, displays punctate foci of DAB signal within the nuclei as it localizes to the centromere of the congregated chromatids (DiB Fig. 2F). In contrast, the developing heart displayed only a few cells with such dot-like pattern at the nuclei. The signal rather localised to regions devoid of methyl green stain in most cardiac cells (Fig. 5A–D). Additionally, we observed exclusively extranuclear SGO1 localisation in adult cerebral cortex alongside the cells that showed its nuclear localisation (DiB Fig. 2E).

2.3. Sgo1 localizes in extranuclear apical region of the epithelial cells and in mesenchyme of the developing gut

X-gal signal at a gross level did not show a noticeable staining above the baseline in the gut; however, sections of the samples revealed highly notable staining patterns obscured by ubiquitous staining. Sgo1 expression was observed in two domains of the gut: at the apical border of epithelial cells and in mesenchymal cells. At E9.5, the X-gal stain strongly localised to epithelial cells and in the surrounding region (Fig. 6A). Interestingly, immunostaining marked SGO1 at the apical area within the cell and outside the methyl green stained nuclei (Fig. 5E). E10.5 embryos showed marked X-gal signal in the epithelial and mesenchymal cells (Fig. 6B). Immunostaining showed strong apical localisation separate from the nuclear stain within the epithelial cells and

Fig. 1. Allele map of Sgo1$^{+/LacZ}$ mice. LacZ cassette insertion was inserted between exon4 and 5 of *Sgo1*. lacZ: β-galactosidase gene, SA: splicing acceptor, pA: polyadenylation sequence. FRT: flippase recognition target, neo: neomycin resistance gene (Koscielny et al., 2014). Multicistronic element is not shown, refer to the genebank file: http://www.mousephenotype.org/data/alleles/MGI:1919665/tm1a(EUCOMM)Wtsi.