**SORBS2 upregulation may contribute to dysfunction in LVNC via Notch pathway**

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SORBS2 upregulation may contribute to dysfunction in LVNC via the Notch pathway

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Running title: SORBS2 upregulation contributes to dysfunction

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Left ventricular noncompaction cardiomyopathy (LVNC) is currently classified as a clinically heterogeneous primary genetic cardiomyopathy by the American Heart Association [1]. It is characterized by increased myocardial trabeculations and recesses. The disease may represent disrupted embryologic development, but its exact embryologic mechanism remains unclear. Sorbin and SH3 domain-containing protein 2 (SORBS2), an adaptor protein, is a member of the SOHO protein family. SORBS2 functions in cytoskeletal organization, cell adhesion, and signaling pathways [2]. SORBS2 was
found to be most highly expressed in the heart at the mRNA and protein levels [3,4]. SORBS2 was also reported to be located at the Z-band and intercalated disk in cultured cardiomyocytes [4]. The Notch signaling pathway is a highly conserved transmembrane receptor protein family in vertebrates and invertebrates. This pathway plays an important role not only in cell and tissue differentiation in multicellular animals but also in the self-renewal of adult cells and tissues. It is essential for the development and tissue homeostasis of multicellular organisms. Notch signaling can promote or inhibit the determination of cell fate, regulate cell proliferation and death, and control the activation of specific differentiation in embryonic and adult self-renewing tissues [5,6]. Previous studies showed that the Notch signaling pathway also plays an important role in the development of the normal cardiovascular system, and changes in this pathway are associated with a series of autosomal dominant inherited human congenital heart diseases, which can lead to human cardiovascular disease [6]. Notch is involved in almost every aspect of cardiogenesis, including the regulation of cardiac fate, original cardiac vascular pattern, and cardiac structural morphogenesis. The Notch pathway has been reported to be an important factor in the regulation of cardiac development in human embryos [7,8]. Hypohaploidy or reduced gene dose of core components of the Notch pathway may lead to congenital heart disease or affect heart development [9].

In the present study, we found for the first time that overexpression of SORBS2 can cause changes in the Notch pathway, which prompted us to investigate whether elevated myocardial SORBS2 is involved in the pathogenesis of LVNC and the underlying molecular mechanisms.

Our previous reports indicated that the expression of SORBS2 is significantly increased in the left ventricular myocardial tissue of LVNC patients, suggesting that the distribution and dysfunction of this protein may be involved in the pathogenesis of LVNC [10]. Therefore, we planned to further screen genes that are functionally related to SORBS2 at the cellular level and construct a vector for the overexpression of SORBS2 (SORBS2-flag-GFP). The differences between the overexpression vector and unloaded vector could be detected by a chip to screen out specific genes (Supplementary Figure S1A) and related enriched pathways. We used chip technology to screen the enriched pathways of the SORBS2 overexpression group (Supplementary Figure S1B). Myocardial cells overexpressing SORBS2 were compared with cells without the SORBS2 gene, and the differentially enriched
pathways were screened out. This led to the identification of the Notch signaling pathway (Supplementary Figure S1C).

Upon screening out differentially expressed genes based on the microarray results (Figure 1A), we focused on several genes in the Notch pathway expressed in cardiac trabeculae, including Anf, Bmp10, Cx40, Tbx20, and Hey2. These genes were verified by western blot analysis, and their trends of variation were consistent with our chip screening results. The key proteins in the Notch pathway, Tbx20 and Hey2, were highly expressed in SORBS2-overexpressing cardiomyocytes, while Anf, Bmp10, and Cx40 were underexpressed (Figure 1B,C).

To verify our previous prediction that CX40 in the Notch pathway may be regulated through SORBS2 interaction, immunofluorescence microscopy was used to detect the localization of CX40 protein expression in cardiomyocytes overexpressing SORBS2 (Figure 2A). The results of epidemic coprecipitation showed that Cx40 interacted with SORBS2 (Figure 2B). In addition, myocardial fibers were also disturbed in SORBS2-overexpressing cardiomyocytes (Figure 2C,D).

We next conducted in vivo studies in mice to further explore how SORBS2 drives the pathogenesis of LVNC. Adeno-associated virus 9 (AAV9)-mediated overexpression of SORBS2 in the cardiac ventricle tissues showed consistent results with our experiments in heart transplant tissue samples and hESC-derived cardiomyocytes (hESC CM). Specifically, we used AAV9 to overexpress SORBS2 in the cardiac left ventricle tissues of wild-type mice and assessed various cardiac phenotypes. Masson trichrome staining of the cardiac left ventricle tissues of the SORBS2-overexpressing mice revealed obvious fibrosis (Supplementary Figure S2A). These in vivo results provide further support to our inferences about SORBS2’s pathomechanistic contribution to LVNC drawn from the observations of LVNC human heart tissue.

In summary, our study based on microarray analysis showed that SORBS2 accumulates in hESC-derived CMs, which affects the Notch pathway. Cx40, as a cointeracting protein and gap-linking protein, may disrupt LVNC-affected hearts. We also showed that in the left ventricular tissues of mice overexpressing SORBS2, the ejection fractions were decreased with microtubule densification. Based on our previous results and existing research reports, we propose that SORBS2 may be involved in the pathogenesis of LVNC by mediating the Notch signaling pathway (Supplementary Figure S3). Myocardial SORBS2 interacts with Cx40 and participates
in the Notch pathway, which results in myocardial fibrosis and heart function impairment. Our findings thus reveal that SORBS2 is involved in myocardial fibrosis and may be useful as a target for the early diagnosis of LVNC.

Supplementary Data
Supplementary Data is available at Acta Biochimica et Biophysica Sinica online.

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Conflict of Interest
The authors declare that they have no conflict of interest.

References
4. B. Wang, E.A. Golemis, G.D. Kruh, ArgBP2, a multiple Src homology 3 domain-containing, Arg/Abl-interacting protein, is phosphorylated in v-Abl-transformed


Figure legends

Figure 1. Chip sequencing results and verification  (A) Analysis of genes differentially expressed between the SORBS2-overexpressing group and the control group (we focused on downregulated Anf, Bmp10, and Cx40 and upregulated Tbx20 and Hey2 genes in the SORBS2-overexpressing group. Blue means down and red means up). (B) Western blot analysis was used to verify the changes of Anf, Bmp10, Cx40, Tbx20, and Hey2 protein expressions. (C) Statistical diagram of Anf, Bmp10, Cx40, Tbx20, and Hey2 protein expressions in the two groups (n=3 for each group, * P<0.05, **P<0.01). Data are shown as the mean±SEM. Student’s t test was used for
statistical analysis.

**Figure 2. Cx40 interacts with SORBS2** (A) Cx40 protein expression in SORBS2-overexpressing cardiomyocytes was detected by immunofluorescence microscopy, scale bar: 25 μm. (B) Proteins extracted from the abovementioned tissues were left untreated (input), while rabbit IgG was used as a positive and negative control. Anti-SORBS2 antibodies were used for co-IP, while anti-Cx40 antibody was used for western blot analysis. Immunoprecipitation showed that Cx40 interacts with SORBS2. (C,D) Muscle fibers in cardiomyocytes overexpressing SORBS2. In these cardiomyocytes, myocardial muscle fibers became disordered.
Fig. 1

A

B

C

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Fig. 2

A

Control

SORBS2-OE

B

IP: Input IgG SORBS2

blot

Cx40 40KD

IP: Input IgG Cx40

blot

SORBS2 125KD

C

Control

SORBS2-OE

D

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Fig. 2
Supplementary Figure S1. Microarray screening for specific genes  (A) Myocardial cell-specific genes differentially expressed upon overexpression of SORBS2. (B) GO analysis upon overexpression of SORBS2 detected by a chip. (C) The main enriched pathways of SORBS2-overexpressing cardiomyocytes are shown, among which the Notch pathway was mainly studied.
A  
Control  SORBS2-OE

B  

Relative fibrotic area (%)  

Control  SORBS2-OE  **

Fig. S2
• Supplementary Figure S2. The phenotypic changes in SORBS2-overexpressing AAV9 vector mice. Masson trichrome staining showing pathological changes in the cardiac left ventricle tissues of mice (n=3 for each group, **P<0.01; Student’s t-test). Data are shown as the mean ± SEM. Scale bar=50 μm.
• Supplementary Figure S3. SORBS2 participates in the pathogenesis of LVNC through the Notch pathway.