論文の内容の要旨

論文題目　The Functional Analysis of a novel ERK Substrate, MCRIP1
（新規ERK基質分子MCRIP1の生理機能解析）

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MCRIP1 is a recently identified CtBP-binding protein that modulates CtBP-mediated gene silencing by inhibiting interactions between the transcriptional corepressor CtBP and PxDLS-containing transcriptional repressors including ZEB1. Although previous studies have shown that CtBP is involved in a broad range of biological processes such as epithelial mesenchymal transition, cellular metabolism and immune response, the precise role of the MCRIP1-CtBP axis in the regulation of embryonic development has been totally unknown. In this study, to clarify the in vivo function of MCRIP1, *Mcrip1* knockout (MCRIP1-KO) mice was generated and analyzed. My results revealed that MCRIP1 is a key regulator for the robust expression of the surfactant proteins in developing lung and is thus critical for survival of neonatal mice.

The majority of MCRIP1-KO mice died in neonatal stage due to respiratory failure caused by deflation of alveolar sacs. Expression of the surfactant proteins, SP-B and SP-C, which reduce the surface tension of the alveolar air-liquid interface, were significantly repressed at both mRNA and protein levels in MCRIP1-KO lungs, which results in collapse of alveoli and small airways. Since SP-B and SP-C are primarily produced in type II AECs, the decreased expression of these surfactant proteins in MCRIP1-KO lungs might be caused either by their transcriptional repression in type II AECs or by defective development of those cells. In this regard, I confirmed that the expression levels of the early and late differentiation markers of type II AECs (*i.e.*, Muc1 and Abca3, respectively), were not significantly altered in MCRIP1-KO lungs, as compared with their wild-type counterparts. Furthermore, electron microscopy revealed that type II AECs were comparably detected in MCRIP1-KO and wild-type lungs, although the number and size of LBs in type II AECs were greatly decreased in MCRIP1-KO mice. Interestingly, these findings are highly similar to those observed in SP-B null mice. Thus, it is suggested that the genetic ablation of MCRIP1 leads to a significant decrease in SP-B and SP-C production in type II AECs, rather than to differentiation defects within type II AECs.

Insufficient production of surfactant proteins in human newborns leads to a life-threatening condition *called* infant respiratory distress syndrome. Although RDS is usually observed in preterm infants due to their immature lung development, full-term ones carrying germline mutations in genes that are involved in surfactant production and metabolism, can also develop refractory RDS. To date, mutations of the genes encoding SP-B, SP-C, ABCA3, and so on have been identified in some cases of familial RDS, but other causative genes remain to be uncovered. Our results imply that MCRIP1 can be a potential target in human RDS. Further investigation is required to clarify the role of MCRIP1 in the etiology of neonatal respiratory diseases.

Previous reports have shown that the Foxp1 and Foxp2 transcriptional repressors contain the CtBP-binding motif and therefore, in collaboration with CtBP, inhibit the expression of several lung specific genes including SP-C. Furthermore, I found that these repressors also bound to the SP-B promoter, and indeed inhibited its transcriptional activity by recruiting CtBP. However, as shown in this and other studies, both Foxp1 and Foxp2 are highly expressed in lung epithelium including type II AECs, which are responsible for surfactant production. It is unclear how do the surfactant proteins escape from Foxp1/2 and CtBP-mediated transcriptional repression in type II AECs. Importantly, my findings demonstrated that the repression of the surfactant proteins took place only in the absence of MCRIP1. Genetic ablation of endogenous MCRIP1 profoundly inhibited the promoter activity and the expression of SP-B as well as SP-C in the cultured lung cells and in mouse

lung tissues. Conversely, MCRIP1 expression reversed Foxp1/2 and CtBP-mediated repression of SP-B reporter activity. Based on these findings, I propose the following model for MCRIP1 function during lung development. In normal, MCRIP1 wild type, lung epithelial cells, MCRIP1 tightly associates with CtBP via its PxDLS motif and competitively inhibits the interactions between CtBP and the DNA-binding transcriptional repressors Foxp1/2. MCRIP1-mediated inhibition of CtBP-Foxp1/2 interactions prevents the recruitment of CtBP to the promoter regions of SP-B and SP-C, thereby maintaining the associated chromatin in a transcriptionally active state to ensure the robust expression of the surfactant proteins. In contrast, without the interference of MCRIP1 in MCRIP1-KO lung epithelium, the freed CtBP can interact with Foxp1/2 bound to the SP-B and SP-C promoters. When recruited to the promoters, the CtBP corepressor complex, which contains chromatin-remodeling enzymes such as HDACs and HMTs, modulates the chromatin conformation through histone deacetylation and methylation, leading to transcriptional silencing of SP-B and SP-C and the resulting respiratory failure. These findings revealed a molecular mechanism for how the production of the surfactant proteins, which are essential for lung function and postnatal survival, is protected from inadvertent gene silencing during embryonic development.