Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Hum Mutat. Author manuscript; available in PMC 2022 June 01.

Published in final edited form as:

Hum Mutat. 2021 June ; 42(6): 694–698. doi:10.1002/humu.24198.

Lung-specific distant enhancer *cis*-regulates expression of *FOXF1* and lncRNA *FENDRR*

Przemyslaw Szafranski[1], **Tomasz Gambin**[1,2,3], **Justyna A. Karolak**[4], **Edwina Popek**[5], **Paweł Stankiewicz**[1]

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas [2]Institute of Computer Science, Warsaw University of Technology, Warsaw, Poland [3]Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland [4]Chair and Department of Genetics and Pharmaceutical Microbiology, Poznań University of Medical Sciences, Poznań, Poland [5]Department of Pathology and Immunology, Baylor College of Medicine, Houston, Texas

Abstract

The *FOXF1* gene, causative for a neonatal lethal lung developmental disorder ACDMPV, maps 1.7-kb away from the long noncoding RNA gene *FENDRR* on the opposite strand, suggesting they may be co-regulated. Using RNA-seq in lung tissue from ACDMPV patients with heterozygous deletions of the *FOXF1* distant enhancer located 286-kb upstream, leaving *FOXF1* and *FENDRR* intact, we have found that the *FENDRR* and *FOXF1* expressions were reduced by approximately 75% and 50%, respectively, and were mono-allelic from the intact chromosome 16q24.1. In contrast, ACDMPV patients with *FOXF1* SNVs had bi-allelic *FENDRR* expression reduced by 66–82%. Corroboratively, depletion of *FOXF1* by siRNA in lung fibroblasts resulted in a 50% decrease of *FENDRR* expression. These data indicate that *FENDRR* expression in the lungs is regulated both in *cis* by the *FOXF1* distant enhancer and in *trans* by FOXF1. Our findings are compatible with an involvement of *FENDRR* in *FOXF1*-related disorders, including ACDMPV.

Correspondence: Paweł Stankiewicz, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, pawels@bcm.edu.

AUTHOR CONTRIBUTIONS

P.Sz. and J.A.K. performed the experiments, T.G. analyzed RNA-seq data, P.Sz. T.G., J.A.K., and P.St. interpreted the data, P.Sz. drafted the manuscript; E.P. histologically verified ACDMPV cases, all the authors critically reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

WEB RESOURCES

ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/
ENCODE, https://www.encodeproject.org/
GeneCards, http://www.genecards.org
GTEx portal, https://gtexportal.org/home/

DATA AVAILABILITY STATEMENT

The sequence variant data was submitted to the ClinVar database (SUB9101558). The ClinVar accessions for this submission are SCV001480485 and SCV001480486.
Data available on request from the authors.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Keywords

Divergent genes; lncRNA enhancer; CNV deletion; SNV; vascular development; congenital lung disorder

Heterozygous single nucleotide variants (SNVs) in *FOXF1* on chromosome 16q24.1 or copy-number variant (CNV) deletions involving *FOXF1* or its distant enhancer, located ~ 286 kb upstream, have been found causative in 80–90% of patients with a neonatal lethal lung developmental disorder Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACDMPV; MIM# 265380) (Stankiewicz et al., 2009; Szafranski et al., 2013, 2016, 2019). Interestingly, unlike SNVs, CNV deletions arise *de novo* almost exclusively on maternal chromosome 16. Approximately 1.7 kb upstream to *FOXF1*, maps the divergently oriented *FOXF1* Adjacent Non-coding Developmental Regulatory RNA (*FENDRR*) gene (MIM# 614975). The interval in between harbors the putative promoters of *FENDRR* and *FOXF1* (hg38 coordinates chr16:86,508,876–86,508,935 and 86,510,478–86,510,537, respectively; Eukaryotic Promoter Database, http://epd.vital-it.ch). Such gene arrangement suggests their co-regulation and a potential involvement of *FENDRR* in the etiology of ACDMPV or other *FOXF1*-related disorders. Interestingly, single umbilical artery and severe cardiac defects, e.g. hypoplastic left heart syndrome, have been observed, with one exception (Bourque et al. 2019), in ACDMPV neonates only with CNV deletions harboring *FOXF1* and *FENDRR* (Szafranski et al. 2016). In support of this notion, *Fendrr* was shown to play an essential role in development of the murine heart, gastrointestinal tract, and lungs (Grote et al., 2013; Lai et al., 2015; Sauvageau et al., 2013). Transcriptome sequencing from different tissue samples obtained from 1043 normal adult individuals showed that *FENDRR* has a restricted expression pattern with the highest level in lungs and urinary bladder, gall bladder, esophagus, prostate, and intestines (Fagerberg et al., 2014; GTEx Consortium, 2013). Moreover, changes in *FENDRR* expression (usually its decrease) correlated with an onset and/or progression of invasive carcinomas, including lung cancer (Chang et al., 2020; Gong et al. 2019; Liang et al., 2017; Miao et al. 2016; Xu & Han, 2019; Yang et al., 2018; Zhang et al., 2018; Zhang et al., 2019; Zhu et al., 2012), gastric cancer (He et al., 2018; Xu et al., 2014; Yin et al., 2019), fibrosis (Geng & Guan, 2017; Gong et al. 2020; Huang et al., 2020), and other disorders (GeneCards at http://www.genecards.org) (Szafranski & Stankiewicz, 2021). However, the mechanisms of the regulation of *FENDRR* expression remain incompletely understood.

Reported here studies involving patient-derived materials (Supp. Table S1) were approved by the IRB for Human Subject Research at Baylor College of Medicine (Protocol H-8712). DNA and RNA extraction, DNA sequencing by the Sanger method, RNA sequencing (RNA-seq), array comparative genomic hybridization, siRNA-based gene silencing, and transcript quantification by real-time PCR (RT-qPCR) were done as described in Materials and Methods (Supporting Information).

The proximity of the *FENDRR* and *FOXF1* promoters and the involvement of both genes in early mammalian development raised a question whether *FOXF1* distant enhancer might be also involved in regulation of the *FENDRR* expression. To explore this possibility, using

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

RNA-seq, we have measured the *FENDRR* transcript levels in lung samples from patients with heterozygous maternal (ACDMPV pts 60.4, 64.5, 155.3) and paternal (pt 179.3) CNV deletions of the *FOXF1* core enhancer (chr16:86,218,986–86,224,837, hg38), leaving *FOXF1* and *FENDRR* intact (Supp. Table S1) and in three age-matched normal lung samples. We have found that the presence of heterozygous enhancer deletions was associated with an ~ 50% decrease of *FOXF1* and an ~ 75% decrease of the *FENDRR* level (based on mean expression values for groups of cases that were used for comparisons), suggesting that *FENDRR* is also regulated by the distant lung-specific enhancer (Figure 1A).

Importantly, we have also found that when the maternal allele of the enhancer was deleted, all informative *FENDRR* and *FOXF1* transcript-specific SNVs originated only from the paternal allele and, *vice versa*, they were only from the maternal *FENDRR* and *FOXF1* allele when the paternal allele of the enhancer was deleted (Figure 1B, Supp. Table S2). Similarly, the residual transcription from the putatively bidirectional *FENDRR* promoter originated from the paternal allele of the promoter when the maternal allele of the enhancer was deleted (pt 60.4; Supp. Figure S1A–C). We have confirmed mono-allelic *FENDRR* expression in ACDMPV patients using Sanger sequencing of a randomly selected SNPs in pts 60.4 and 179.3 cDNA (Supp. Figure S2). In contrast, the expression pattern of *FENDRR* and *FOXF1* was bi-allelic when both parental alleles of the enhancer were present, *i.e.*, in normal lungs (Supp. Figure S3, Supp. Tables S2, S3), in the lungs of ACDMPV patients with the *FOXF1* heterozygous frame-shifting variant (pt 123.3) (Supp. Figure S4, Supp. Tables S2, S3), or the missense variant (pt 77.3) (Supp. Figure S5, Supp. Table S2). Thus, the regulatory region, originally identified as the lung-specific *FOXF1* distant enhancer, functions also as a strong lung-active *FENDRR* enhancer in *cis*, fulfilling the criteria of an enhancer (Gasperini et al., 2020). We and others have previously shown by chromosome conformation capture analyses that this enhancer physically interacts with *FENDRR-FOXF1* intergenic region in humans (Szafranski et al., 2013) and mice (Seo et al., 2016). It is possible that different portions of the *FENDRR-FOXF1* enhancer interact only with the *FENDRR* or *FOXF1* promoters, or the enhancer as a whole interacts with both promoters interchangeably or the two promoters share common interaction site with the enhancer.

The presence of the pathogenic SNVs in *FOXF1* correlated with a 66–82% decrease of the bi-allelic *FENDRR* expression (Figure 1A), although RNA from only two ACDMPV individuals with variants in *FOXF1* was available. To further verify the potential FOXF1 involvement in the regulation of *FENDRR*, we have generated transient *FOXF1* knockdowns in fetal lung fibroblasts IMR-90 using siRNAs targeting both exons of *FOXF1*. Using RT-qPCR, we have found that the decrease of *FOXF1* transcript by ~ 85% resulted in ~ 50% reduction of the *FENDRR* transcript level (Figure 2A). *FENDRR* decrease in *FOXF1*-depleted lung fibroblasts corroborated also previous findings in the conditional *Foxf1* knockout mice (Ren et al., 2014), however, the interpretation of the mouse experiment was to some point hampered by the fact that the knockout procedure involved targeting the *Foxf1* promoter and might have interfered with functioning of the *Fendrr* promoter.

In contrast, siRNA knockdown of *FENDRR* in IMR-90 fibroblasts by ~ 85% had no significant effect on the expression of *FOXF1* (Figure 2B), consistent with the data in the *Fendrr* knockout mice generated by Sauvageau et al. (2013). In a different *Fendrr* knockout

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

mice, Grote et al., (2013) showed an increased expression of *Foxf1* in the heart. However, this increase could result from the knockout construct with a replacement of the 1st exon of *Fendrr* with a strong transcriptional stop signal.

Since no ChIP-seq or other data on FOXF1 binding to chr16q24.1 in human lung cells are available, we explored the possibility of an indirect contribution of FOXF1 to *FENDRR* expression. We have analyzed our ACDMPV RNA-seq data for changes in the expression of more than 30 transcription regulators that were previously identified by ChIP-seq (ENCODE) as binding within the *FENDRR* promoter or enhancer region and thus having a potential to regulate *FENDRR* expression. We have found that the expression of the histone methyltransferase subunit gene, *ASH2L*, was reduced by ~ 40% in ACDMPV lung samples, correlating with reduced expression of *FOXF1* and *FENDRR* (Supp. Figure S1D).

In summary, we demonstrate that *FENDRR* expression in human lungs is regulated by the distant lung-specific *FOXF1* enhancer in *cis* and by FOXF1 transcription factor (directly or indirectly) in *trans*. These findings also suggest the potential involvement of *FENDRR* in etiology of some FOXF1-associated disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding information

NIH/NHLBI grant R01HL137203 to P.St., National Science Centre in Poland grant 2019/35/D/NZ5/02896 to J.A.K., and, in part, NIH grants S10 1S10OD023469 and P30CA125123 to the Genomic and RNA Profiling Core at Baylor College of Medicine.

References

Bourque DK, Fonseca IC, Staines A, Teitelbaum R, Axford MM, Jobling R, …, Chitayat D (2019). Alveolar capillary dysplasia with misalignment of the pulmonary veins and hypoplastic left heart sequence caused by an in frame deletion within *FOXF1*. American Journal of Medical Genetics A, 179(7), 1325–1329.

Chang Y, Xue X, Li C, Zhao W, Ma Y, Xu F, … Chen L (2020). MIR205HG facilitates carcinogenesis of lung squamous cell carcinoma in vitro revealed by long noncoding RNA profiling. Acta Biochimica et Biophysica Sinica (Shanghai), 2(4), 371–381.

Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, … Uhlén M (2014). Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Molecular & Cellular Proteomics, 13(2), 397–406.

Gasperini M, Tome JM, & Shendure J (2020). Towards a comprehensive catalogue of validated and target-linked human enhancers. Nature Reviews Genetics, 21(5), 292–310.

Geng H, & Guan J (2017). MiR-18a-5p inhibits endothelial-mesenchymal transition and cardiac fibrosis through the Notch2 pathway. Biochemical and Biophysical Research Communications, 491(2), 329–336. [PubMed: 28733035]

Gong F, Dong D, Zhang T, & Xu W (2019). Long non-coding RNA FENDRR attenuates the stemness of non-small cell lung cancer cells via decreasing multidrug resistance gene 1 (MDR1) expression through competitively binding with RNA binding protein HuR. European Journal of Pharmacology, 853, 345–352. [PubMed: 30981768]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Gong L, Zhu L, & Yang T (2020). Fendrr involves in the pathogenesis of cardiac fibrosis via regulating miR-106b/SMAD3 axis. Biochemical and Biophysical Research Communications, 524(1), 169–177. [PubMed: 31982134]

Grote P, Wittler L, Hendrix D, Koch F, Währisch S, Beisaw A,… Herrmann BG (2013). The tissue-specific lncRNA *Fendrr* is an essential regulator of heart and body wall development in the mouse. Developmental Cell, 24(2), 206–214. [PubMed: 23369715]

GTEx Consortium. (2013). The Genotype-Tissue Expression (GTEx) project. Nature Genetics, 45, 580–585. [PubMed: 23715323]

He Z, Wang X, Huang C, Gao Y, Yang C, Zeng P, & Chen Z (2018). The FENDRR/miR-214–3P/TET2 axis affects cell malignant activity via RASSF1A methylation in gastric cancer. American Journal of Translational Research, 10(10), 3211–3223. [PubMed: 30416662]

Huang C, Liang Y, Zeng X, Yang X, Xu D, Gou X, … Liu L (2020). Long noncoding RNA FENDRR exhibits antifibrotic activity in pulmonary fibrosis. American Journal of Respiratory Cell and Molecular Biology, 62(4), 440–453. [PubMed: 31697569]

Lai KM, Gong G, Atanasio A, Rojas J, Quispe J, Posca J, … Valenzuela DM (2015). Diverse phenotypes and specific transcription patterns in twenty mouse lines with ablated lincRNAs. PLoS One, 10, e0125522. [PubMed: 25909911]

Liang C, Zhang X, Wang HM, Liu XM, Zhang XJ, Zheng B, …., Ma ZL (2017). MicroRNA-18a-5p functions as an oncogene by directly targeting IRF2 in lung cancer. Cell Death & Disease, 8, e2764. [PubMed: 28471447]

Miao L, Huang Z, Zengli Z, Li H, Chen Q, Yao C, … Wang Y (2016). Loss of long noncoding RNA FOXF1-AS1 regulates epithelial-mesenchymal transition, stemness and metastasis of non-small cell lung cancer cells. Oncotarget, 7(42), 68339–68349. [PubMed: 27577075]

Ren X, Ustiyan V, Pradhan A, Cai Y, Havrilak JA, Bolte CS, … Kalinichenko VV (2014). FOXF1 transcription factor is required for formation of embryonic vasculature by regulating VEGF signaling in endothelial cells. Circulation Research, 115(8), 709–720. [PubMed: 25091710]

Sauvageau M, Goff LA, Lodato S, Bonev B, Groff AF, Gerhardinger C, … Rinn JL (2013). Multiple knockout mouse models reveal lincRNAs are required for life and brain development. Elife, 2, e01749. [PubMed: 24381249]

Seo H, Kim J, Park GH, Kim Y, & Cho SW (2016). Long-range enhancers modulate *Foxf1* transcription in blood vessels of pulmonary vascular network. Histochemistry and Cell Biology, 146(3), 289–300. [PubMed: 27166834]

Stankiewicz P, Sen P, Bhatt SS, Storer M, Xia Z, Bejjani BA, … Shaw-Smith C (2009). Genomic and genic deletions of the FOX gene cluster on 16q24.1 and inactivating mutations of *FOXF1* cause alveolar capillary dysplasia and other malformations. American Journal of Human Genetics, 84(6), 780–791. [PubMed: 19500772]

Szafranski P, Dharmadhikari AV, Brosens E, Gurha P, Kolodziejska KE, Zhishuo O, … Stankiewicz P (2013). Small noncoding differentially methylated copy-number variants, including lncRNA genes, cause a lethal lung developmental disorder. Genome Research, 23(1), 23–33. [PubMed: 23034409]

Szafranski P, Gambin T, Dharmadhikari AV, Akdemir KC, Jhangiani SN, Schuette J, … Stankiewicz P (2016). Pathogenetics of alveolar capillary dysplasia with misalignment of pulmonary veins. Human Genetics, 135(5), 569–586. [PubMed: 27071622]

Szafranski P, Liu Q, Karolak JA, Song X, de Leeuw N, Faas B, … Stankiewicz P (2019). Association of rare non−coding SNVs in the lung−specific *FOXF1* enhancer with a mitigation of the lethal ACDMPV phenotype. Human Genetics, 138(11–12), 1301–1311. [PubMed: 31686214]

Szafranski P, & Stankiewicz P (2021). Long Non-Coding RNA FENDRR: Gene Structure, Expression, and Biological Relevance. Genes, 12(2): 177. [PubMed: 33513839]

Xu TP, Huang MD, Xia R, Liu XX, Sun M, Yin L, … Shu YQ (2014). Decreased expression of the long non-coding RNA FENDRR is associated with poor prognosis in gastric cancer and FENDRR regulates gastric cancer cell metastasis by affecting fibronectin1 expression. Journal of Hematology & Oncology, 7, 63. [PubMed: 25167886]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Xu R, & Han Y (2019). Long non-coding RNA FOXF1 adjacent non-coding developmental regulatory RNA inhibits growth and chemotherapy resistance in non-small cell lung cancer. Archives of Medical Science, 15(6), 1539–1546. [PubMed: 31749883]

Yang L, Wu D, Chen J, Chen J, Qiu F, Li Y, … Lu J (2018). A functional CNVR_3425.1 damping lincRNA FENDRR increases lifetime risk of lung cancer and COPD in Chinese. Carcinogenesis, 39(3), 347–359. [PubMed: 29293945]

Yin SL, Xiao F, Liu YF, Chen H, & Guo GC (2019). Long non-coding RNA FENDRR restrains the aggressiveness of CRC via regulating miR-18a-5p/ING4 axis. Journal of Cellular Biochemistry, 121(8–9), 3973–3985.

Zhang MY, Zhang ZL, Cui HX, Wang RK, & Fu L (2018). Long non-coding RNA FENDRR inhibits NSCLC cell growth and aggressiveness by sponging miR-761. European Review for Medical Pharmacological Sciences, 22(23), 8324–8332. [PubMed: 30556873]

Zhang G, Wang Q, Zhang X, Ding Z, & Liu R (2019). LncRNA FENDRR suppresses the progression of NSCLC *via* regulating miR-761/TIMP2 axis. Biomedicine & Pharmacotherapy, 118, 109309. [PubMed: 31545237]

Zhu X, Li H, Long L, Hui L, Chen H, Wang X, … Xu W (2012). miR-126 enhances the sensitivity of non-small cell lung cancer cells to anticancer agents by targeting vascular endothelial growth factor A. Acta Biochimica et Biophysica Sinica, 44, 519–526. [PubMed: 22510476]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

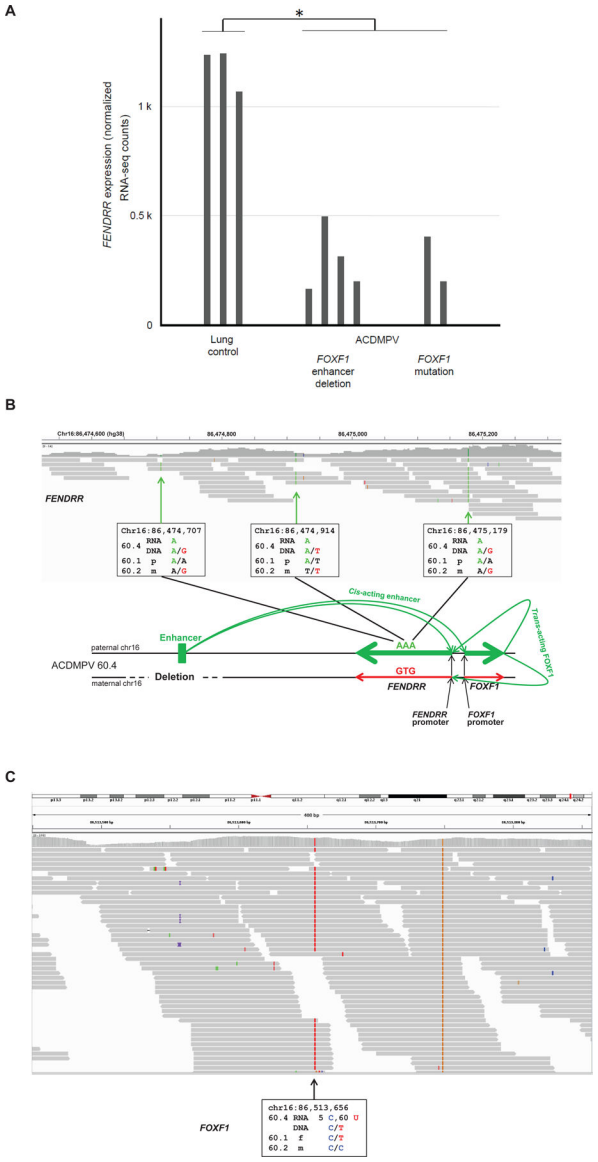

Figure 1. Regulation of the *FENDRR* transcription.

(**A**) The decrease of the *FENDRR* levels in the lungs of ACDMPV pts 64.5, 60.4, 155.3, 179.3 (enhancer deletions), 73.3, and 123.3 (*FOXF1* SNVs) (One-way ANOVA with post-hoc Tukey HSD test $*P < 0.01$). Mono-allelic expression of (**B**) *FENDRR* and (**C**) *FOXF1* from the paternally inherited chromosome 16 in the presence of the heterozygous CNV deletion of the distant lung-specific *FOXF1* enhancer on the maternally inherited chromosome 16 (pt 60.4). Vertical lines on RNA-seq reads (grey bars) represent adenines (green) or uracil (red). Schematic drawing below the sequence reads is shown not to scale. Abbreviations: p, paternal; m, maternal.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

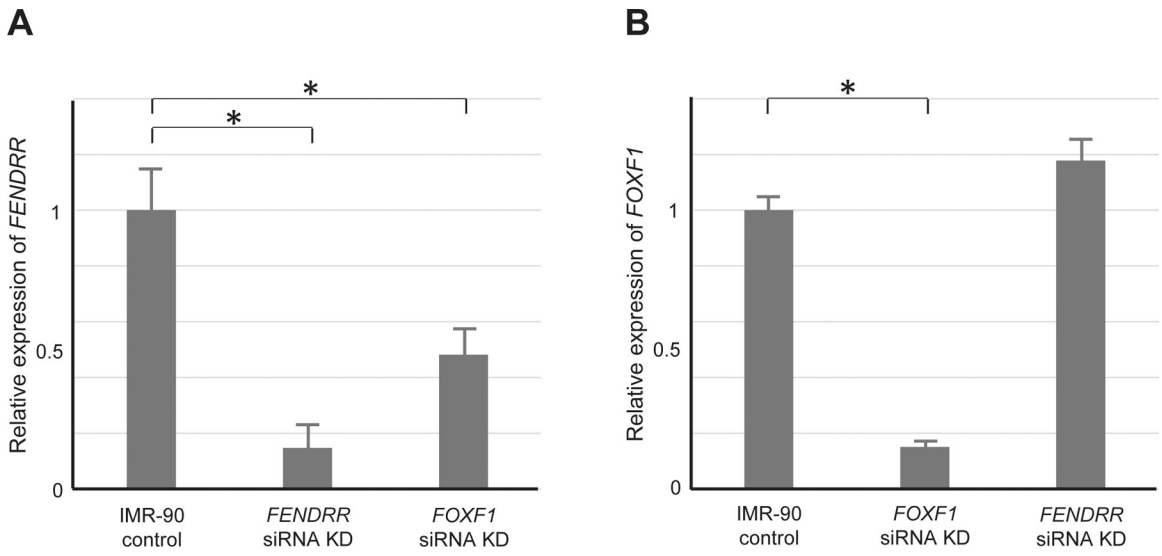

Figure 2.

(**A**) RNAi-based gene silencing in IMR-90 lung fibroblasts showing that the depletion of the *FOXF1* transcript correlates with the 2.1±0.3 fold decrease of *FENDRR* expression. (**B**) Loss of *FENDRR* does not have a significant effect on *FOXF1* expression. The plotted data represent average values from experiments performed in triplicate ± SD (**P* < 0.01).