

RAPID REPORT | Translational Physiology

miR-142-3p is associated with aberrant WNT signaling during airway remodeling in asthma

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Bartel S, Carraro G, Alessandrini F, Krauss-Etschmann S, Ricciardolo FL, Bellusci S. *miR-142-3p* is associated with aberrant WNT signaling during airway remodeling in asthma. *Am J Physiol Lung Cell Mol Physiol* 315: L328–L333, 2018. First published May 3, 2018; doi:10.1152/ajplung.00113.2018.—Asthma is characterized by a chronic inflammation and remodeling of the airways. Although inflammation can be controlled, therapeutic options to revert remodeling do not exist. Thus, there is a large and unmet need to understand the underlying molecular mechanisms to develop novel therapies. We previously identified a pivotal role for *miR-142-3p* in regulating airway smooth muscle (ASM) precursor cell proliferation during lung development by fine-tuning the Wingless/Integrase I (WNT) signaling. Thus, we here aimed to investigate the relevance of this interaction in asthma. We performed quantitative RT-PCR and immune staining in a murine model for ovalbumin-induced allergic airway inflammation and in bronchial biopsies from patients with asthma and isolated primary fibroblasts thereof. *miR-142-3p* was increased in hyperproliferative regions of lung in murine and human asthma, whereas this microRNA (miRNA) was excluded from regions with differentiated ASM cells. Increases in *miR-142-3p* were associated with a decrease of its known target *Adenomatous polyposis coli*. Furthermore, we observed a differential expression of *miR-142-3p* in bronchial biopsies from patients with early or late onset severe asthma, which coincided with a differential WNT signature. Our data suggest that *miR-142-3p* is involved in regulating the balance between proliferation and differentiation of ASM cells in asthma, possibly via controlling WNT signaling. Thus, this miRNA might be an interesting target to prevent ASM hyperproliferation in asthma.

airway remodeling; asthma; miR-142-3p; WNT

INTRODUCTION

Western countries have experienced a large rise in chronic inflammatory diseases such as type 1 diabetes, inflammatory bowel disease, and asthma (2) over the last decades, and asthma currently affects around 235 million people worldwide (25). Available treatments can only relieve symptoms, and thus

asthma represents a huge burden for health care systems. Accordingly, there is a large need to develop novel therapeutic approaches.

Asthma is characterized by chronic inflammation, hyperresponsiveness, and remodeling of the airways. The latter is particularly important because it may develop in childhood (19) but usually does not respond to therapy. Hallmarks of airway remodeling include goblet cell metaplasia, basement membrane thickening, and hyperproliferation of airway smooth muscle (ASM) cells around the airways (reviewed in Ref. 10).

The molecular mechanisms driving airway remodeling in asthma are far from being understood, but there are first hints that microRNAs (miRNAs) might be critically involved (20). These short, noncoding RNAs can posttranscriptionally regulate target gene expression and thus represent a dynamic regulatory fine-tuning of pathways (7). miRNAs also have been shown to be involved in asthma (reviewed in Ref. 15).

In previous work, we identified dysregulated miRNAs in lung tissue of mice with allergic airway inflammation (AAI), and *miR-142-3p* was one of the most highly upregulated miRNAs (4). *miR-142-3p* has been previously described for fine-tuning the balance between proliferation and differentiation of ASM progenitor cells during lung development (6) by targeting *Adenomatous polyposis coli* (*Apc*), a negative regulator of the Wingless/Integrase-1 (WNT) pathway (12).

Therefore, we hypothesized that *miR-142-3p* is involved in controlling proliferation and differentiation of ASM cells during asthma pathogenesis by fine-tuning WNT signaling. Accordingly, we investigated this in lung tissue of a murine AAI model and bronchial biopsies from human patients with mild or severe asthma.

MATERIAL AND METHODS

Animals. Female Balb/c J mice (Charles River, Sulzfeld, Germany) were housed in individually ventilated cages with ad libitum access to a standard pellet diet and water. All experiments were conducted under German federal guidelines for the use and care of laboratory animals and were approved by the Government of the District of Upper Bavaria (AZ 55.2-1-54-2531-49-07).

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Induction of AAI in mice. AAI was induced as previously described (4). Briefly, mice were sensitized six times intraperitoneally with 1 μ g ovalbumin (OVA) in alum (or PBS in alum) and challenged with 1% OVA aerosol on day 70 and 71 after first sensitization. AAI was verified by confirmation of OVA-specific IgE, inflammatory (eosinophilic) cell infiltration, and goblet cell metaplasia (4).

RNA extraction. Total RNA including small RNAs was isolated by using the microRNaseasy micro kit (Qiagen Benelux BV, Venlo, The Netherlands) according to the manufacturer's instructions and quantified and analyzed for purity (presence of protein of phenol) by a NanoDrop ND-1000 (Thermo Fisher Scientific, Waltham, MA). Absence of RNA degradation was evaluated by denaturing gel electrophoresis for murine RNA samples and by checking for equal expression of housekeeping genes between the samples for both human and murine RNA.

Quantitative real-time PCR. Quantitative real-time PCR (qRT-PCR) and respective data analysis was performed as previously described (5). We used a TaqMan RT-PCR system (Thermo Fisher Scientific) for analyzing miRNAs and specific WNT pathway primers (Qiagen) on the LightCycler 480 (Roche Diagnostics, Mannheim, Germany). Data was normalized to small control RNA U6 (Thermo Fisher Scientific) for miRNA, and expression differences were calculated with the $\Delta\Delta$ threshold cycle (Ct) method (16).

Immunofluorescence and in situ hybridization. Immunofluorescence (IF) and in situ hybridizations were performed as previously described (6). Briefly, tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned on poly-L-lysine coated slides. Antibodies against APC (Abcam, Cambridge, UK), KI67 (Novabios srl, Limena, Italy), and alpha smooth muscle actin (ACTA2; Sigma Aldrich, Hamburg, Germany) were used at 1:200 dilutions. All IF data was analyzed by trained investigators who did not know the identity of the samples. All adjustments for background and thresholds were performed identically for all images before quantification.

Human samples. We included subjects with mild ($n = 5$) and severe ($n = 16$) asthma, and severe were stratified for early (<18 yr old; $n = 6$) and late onset (>18 yr old; $n = 10$). All patients were identified and treated according to the Global Initiative for Asthma and European Respiratory Society/American Thoracic Society criteria (9), although early and late onset did not differ in the dose of inhaled corticosteroids (unpaired t -test). Fiberoptic bronchoscopy was performed as previously described (17). Biopsy samples were either embedded in Tissue Tek II OCT (Miles Scientific, Newark, DE) or directly frozen in liquid nitrogen. Primary cultures of asthmatic bronchial fibroblasts were obtained by enzymatic digestion of bronchial biopsy specimens and characterized as previously described (18).

Ethical statements for human samples. The local ethics committee (San Luigi Hospital: Protocols 1759/2008-14871/2009) approved the study, which conformed to the Declaration of Helsinki; written informed consent was obtained from each subject.

Statistical analysis. All results have been evaluated by using Student's t -test with $*P < 0.05$.

RESULTS

Increase of *miR-142-3p* expression in hyperproliferative regions of the murine lung. To determine a possible association between *miR-142-3p* and ASM cell proliferation or differentiation during asthma pathogenesis, we first evaluated its expression in a murine model for acute OVA-induced AAI (Fig. 1A).

We observed a significant upregulation of *miR-142-3p* in murine lung homogenate of OVA/OVA treated animals versus PBS/OVA controls (Fig. 1B). A subsequent in situ hybridization of lung tissue sections combined with a costaining with KI67 revealed that *miR-142-3p* was increased in hyperproliferative regions in the lung (Fig. 1, C–F). On the other hand,

ApC, the previously reported target for *miR-142-3p* (6), was excluded from those regions (Fig. 1, K–N), whereas a WNT target gene, Myc, was increased in animals with AAI (Fig. 1, G–J).

Activation of WNT signaling in experimental asthma. As *miR-142-3p* has been shown to regulate WNT signaling, we next analyzed the expression of 20 prominent WNT pathway genes in lung homogenate by qRT-PCR. Overall, we observed an activation of WNT signaling in AAI (OVA/OVA) compared with controls (Fig. 1O). In particular, we found a significant increase of *Wnt5a*, *Myc*, *cyclin D1*, *adenovirus early region 1A-associated protein 300*, *Frizzled-5*, and *RuvB-like AAA ATPase* but a decrease of *Wnt11*, *Wnt16*, *Wnt2b*, *Apc*, and *axis inhibition protein 2*.

Inverse regulation of *miR-142-3p* and APC in human asthma. To translate our findings to human asthma, we investigated bronchial biopsies of patients with mild and severe asthma (Table 1) or primary fibroblasts isolated from those.

Staining biopsies with ACTA2 to determine fully differentiated ASM cells revealed more ACTA2⁺ cells in patients with severe asthma (Fig. 2, A–D) compared with patients with mild asthma. *miR-142-3p* expression was decreased in severe asthma (Fig. 2E) and seemed to be inversely correlated with ACTA2 expression and was not co-expressed in the same cell type (Fig. 2, A–D). Furthermore, when we cultured primary fibroblasts isolated from bronchial biopsies, cells were more KI67⁺ when derived from patients with mild asthma (Fig. 2, F–H) but less APC⁺ (Fig. 2, I–K) compared with cells from patients with severe asthma, suggesting a higher proliferative potential.

To investigate possible different molecular endotypes within patients with severe asthma, we stratified patients with severe asthma by an early asthma onset (age <18) or a late asthma onset (age >18). Patients with late onset asthma showed increased levels of *miR-142-3p* in bronchial biopsies (Fig. 2L) compared with patients with early onset.

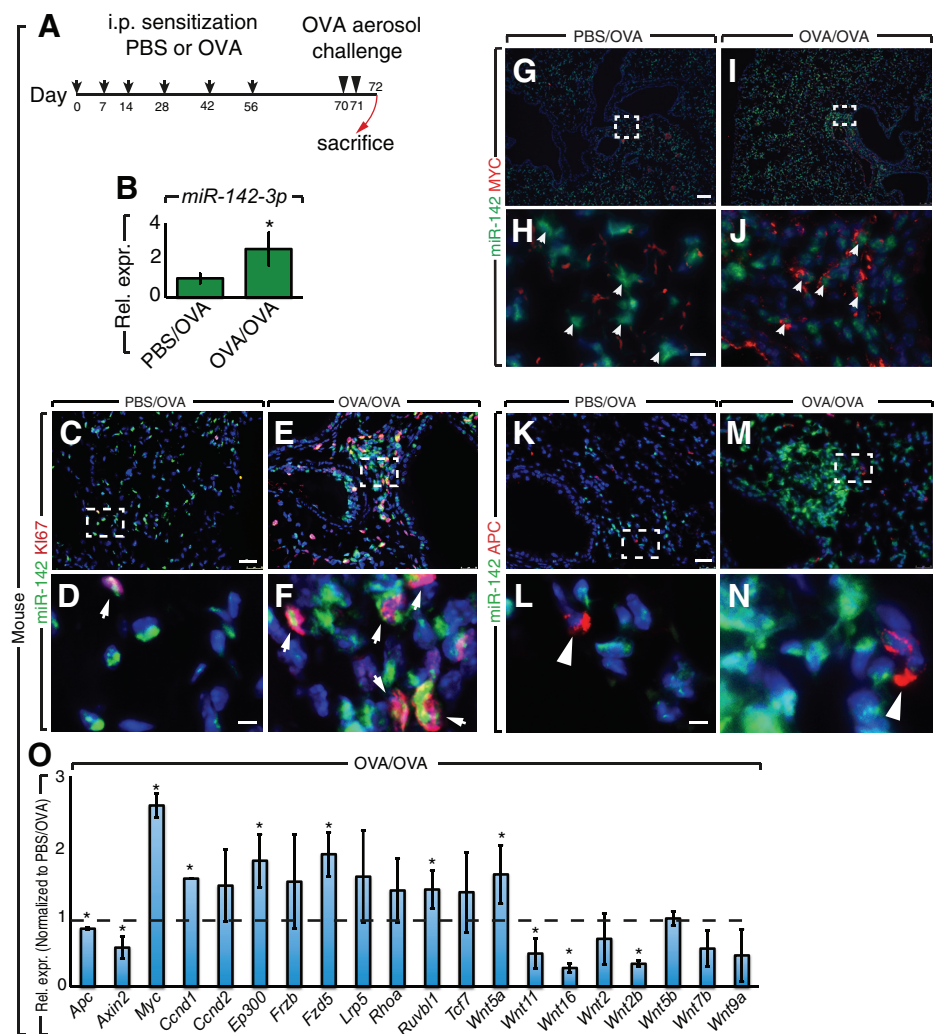
WNT signaling is differentially activated in early versus late onset severe asthma. Because our previous data suggested a role for *miR-142-3p* in the regulation of WNT signaling, we next performed an extensive qRT-PCR-based expression analysis of 78 WNT-associated genes in bronchial biopsies of patients with early or late onset severe asthma. The factors *Frizzled-8*, *MYC*, *JUN*, *segment polarity protein dishevelled homolog DVL1*, *F-box/WD repeat-containing protein 4*, *matrix metalloproteinase 7 (MMP7)*, *Ras homolog family member U*, *WNT inhibitory factor 1*, *WNT16*, *WNT3*, and *WNT9A* were found to be increased in late onset severe asthma compared with early onset (Fig. 3). The WNT ligands *WNT5a* and *WNT6*, and Dickkopf-related protein 1, were significantly higher patients with early onset severe asthma.

DISCUSSION

This study demonstrates an association between *miR-142-3p*, WNT signaling, and hyperproliferative regions in the lung in murine AAI and patients with asthma. Furthermore, we observed for the first time that several WNT-associated factors are differentially expressed in patients with early or late onset severe asthma.

miR-142-3p has already been described in differentiation of stem cells or cardiomyocytes, and in hematopoiesis, immune

Fig. 1. Treatment scheme (A). Quantitative (q)RT-PCR of *miR-142-3p* in murine lung homogenate (B). In situ hybridization for *miR-142-3p* (green) combined with IF for KI67 (red; C–F), MYC (red; G–J), and APC (K–N). Scale bars: 75 μ m (G and I), 25 μ m (C, E, K, and M), and 8 μ m (H and J). qRT-PCR of WNT signaling genes in lung homogenate displayed as relative expression (rel. expr.) of OVA/OVA vs. PBS/OVA (O). All $n = 5$ animals/group, $*P < 0.05$. APC, adenomatous polyposis coli; IF, immunofluorescence; i.p., intraperitoneal; OVA, ovalbumin; WNT, Wingless/Integrase I.



tolerance, or lung cancer (reviewed in Ref. 22). Previously, we found that *miR-142-3p* regulates ASM cell proliferation and differentiation by fine-tuning WNT and FGF signaling during lung mesenchyme development. Briefly, specific targeting of

Apc by *miR-142-3p* in murine embryonic lungs led to activation of WNT signaling and enhanced proliferation of ASM progenitor cells. Upon an *miR-142-3p* knockdown, progenitor cells differentiated into ASM cells because of negative regu-

Table 1. Patient demographics

	All	Mild	Severe	Severe Early Onset	Severe Late Onset
<i>n</i>	1	5	16	6	10
Sex, women/men	11/10	4/1	7/9	3/3	4/6
Atopy, yes/no	10/11	3/2	7/9	6/0	1/9
Smoke, yes/ex-smoker/no	2/9/10	1/2/2	1/8/7	0/2/4	1/5/4
Age, yr	58 $\pm$ 9	51.8 $\pm$ 6	60 $\pm$ 10	56 $\pm$ 11	62 $\pm$ 9
BMI, kg/m ²	25 $\pm$ 3	23.5 $\pm$ 4	26 $\pm$ 3	24.7 $\pm$ 2.5	27 $\pm$ 3.5
FEV1, %pred	72 $\pm$ 19	92 $\pm$ 11	66 $\pm$ 16*	65 $\pm$ 16	66 $\pm$ 17
FVC, %pred	75 $\pm$ 23	92 $\pm$ 11	67 $\pm$ 21**	78 $\pm$ 21	61 $\pm$ 19
FEV1 post, ml	279 $\pm$ 112	267 $\pm$ 80	282 $\pm$ 119	243 $\pm$ 48	305 $\pm$ 145
FeNO	29 $\pm$ 25	35 $\pm$ 30	27.5 $\pm$ 20	28 $\pm$ 26	28 $\pm$ 17
Exacerbation	2 $\pm$ 2	0.5 $\pm$ 0.5	2.4 $\pm$ 1.9*	2.3 $\pm$ 2.1	2.4 $\pm$ 1.9
Beclomethasone HFA	436 $\pm$ 269	150 $\pm$ 50	526 $\pm$ 245*	603 $\pm$ 328	480 $\pm$ 185
Blood eosinophils, cells/ μ l	258 $\pm$ 200	316 $\pm$ 233	240 $\pm$ 194	186 $\pm$ 164	294 $\pm$ 213
Bronchial eosinophils, cells/mm ²	42.8 $\pm$ 30	36 $\pm$ 24	45 $\pm$ 33	45 $\pm$ 26	45 $\pm$ 38
Blood neutrophils, cells/ μ l	4,365 $\pm$ 2,221	3,406 $\pm$ 671	4,665 $\pm$ 2,461	3,723 $\pm$ 871	4,722 $\pm$ 2,621
Bronchial neutrophils, cells/mm ²	70.9 $\pm$ 39	34 $\pm$ 29	82 $\pm$ 36*	88 $\pm$ 28	79 $\pm$ 40

Values are means $\pm$ SD; *n* = no. of patients. BMI, body mass index; FeNO, fraction of exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; HFA, hydrofluoroalkane; %pred, percentage predicted. * $P < 0.05$; ** $P < 0.01$.

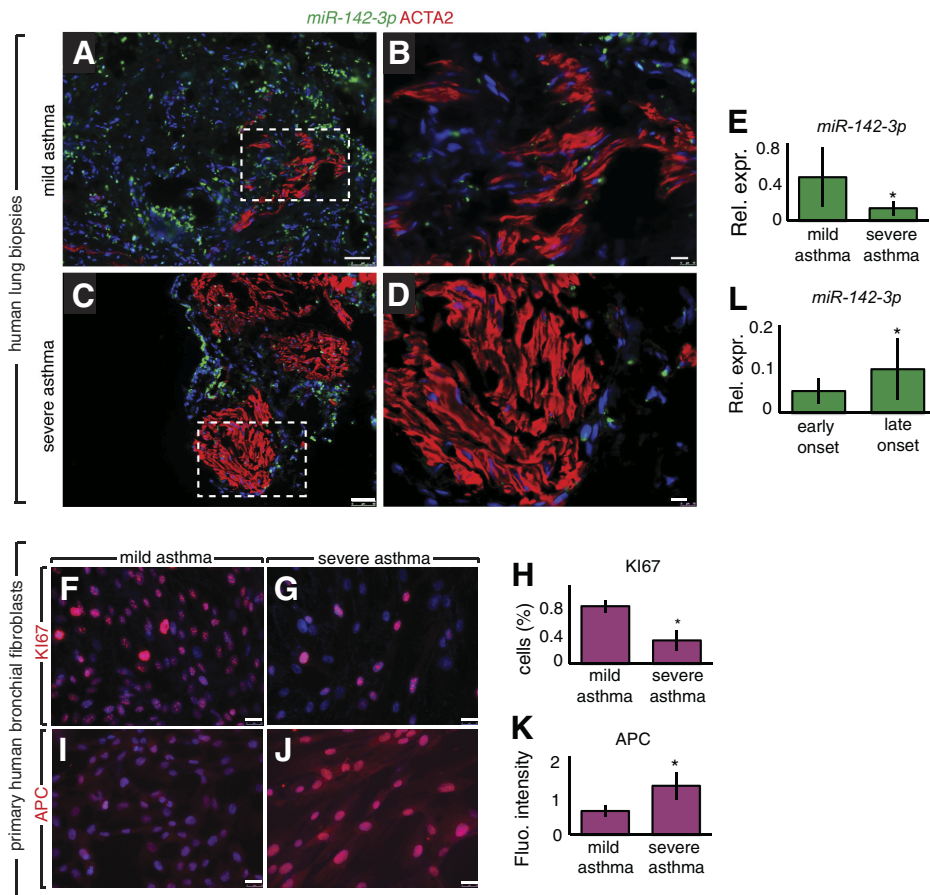


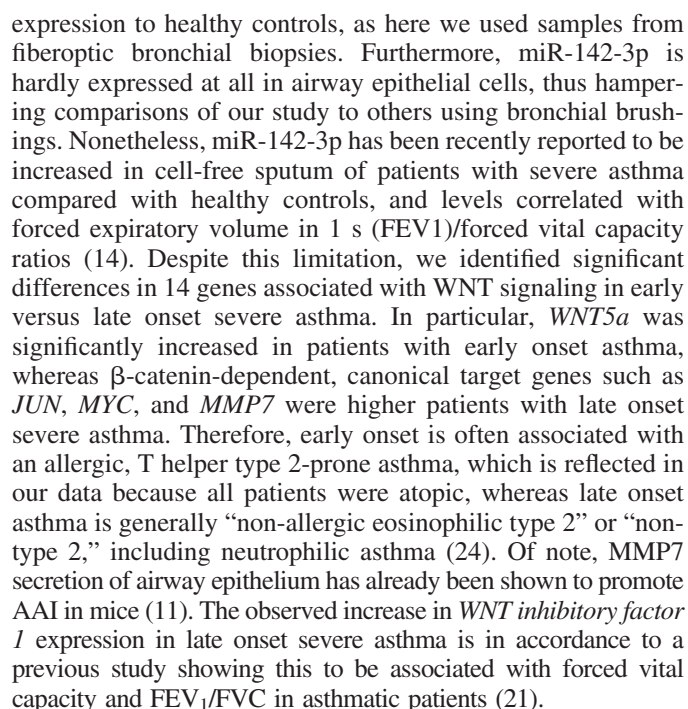
Fig. 2. In situ hybridization for *miR-142-3p* (green) and IF for ACTA2 (red) in bronchial biopsies of patients with mild (A and B) vs. severe (C and D) asthma. Scale bars: 75 μ m (A and B) and 25 μ m (C, D, and F–J). Dashed boxes indicate magnification area for B and D. Representative images of $n = 3$ per group. Quantitative (q)RT-PCR for *miR-142-3p* in frozen bronchial biopsies from patients with mild ($n = 4$) and severe ($n = 8$) asthma (E). IF of primary bronchial fibroblasts for KI67 (red; F–H) and APC (red; I–K) with respective quantification (H and K) mean $\pm$ SD ($n = 3$, $n = 4$), $*P < 0.05$. Scale bar = 25 μ m. qRT-PCR for *miR-142-3p* in bronchial biopsies of patients with early ($n = 5$) vs. late ($n = 4$) onset severe asthma (L). Mean $\pm$ SD, $*P < 0.05$. ACTA2, alpha smooth muscle actin; APC, adenomatous polyposis coli; IF, immunofluorescence; rel. expr., relative expression.

lation of WNT signaling by Apc (6). Here, we now show first hints that fine-tuning of ASM differentiation by *miR-142-3p* might be implicated in asthma, a disease characterized by hyperproliferation of ASM cells leading to airway obstruction. We here report that *miR-142-3p* is mainly expressed in hyperproliferative regions in the murine AAI lung, whereas its target, Apc, was excluded from those. In line with this, APC was significantly decreased in highly proliferating KI67⁺ primary human bronchial fibroblasts of patients with mild asthma, whereas *miR-142-3p* levels were increased in whole biopsies of those patients compared with patients with severe asthma. IF of human bronchial biopsies revealed an increased presence of ACTA2⁺ terminally differentiated ASM cells but a lack of *miR-142-3p* in those regions. Altogether, these data suggest that *miR-142-3p* might be involved in the early initiation of ASM remodeling in asthma by suppressing WNT signaling to initiate the proliferation of ASM progenitor cells. It is intriguing to speculate that in later phases of asthma pathogenesis, possibly because of a loss of *miR-142-3p*, these cells differentiate into ASM cells and contribute to pathology. Therefore, *miR-142-3p* might represent an interesting target for approaches to prevent airway remodeling.

The role of *miR-142-3p* in asthma is presumably related to the simultaneous regulation of several relevant targets; however, here we focused on WNT signaling because this pathway is 1) critical for regulating the balance between proliferation and differentiation of ASM progenitor cells (6) and 2) has been implicated in asthma pathogenesis in several studies before (3,

8, 21, 23). WNT signaling plays a pivotal role in organism development and is in general activated upon binding of a WNT ligand to receptors, such as *Frizzled*, leading to context-dependent transcription of target genes (reviewed in Ref. 1).

Here we validated the *miR-142-3p*-mediated Apc downregulation in proliferative cells of the lung but expanded this to asthma pathogenesis. APC is a negative regulator of β -catenin that induces, upon binding in combination with axis inhibition protein and GSK3B, its ubiquitination and subsequent degradation, thereby preventing β -catenin nuclear translocation and transcription of target genes (1). We observed an increase of typical targets for β -catenin-driven gene expression (canonical WNT signaling) such as *cyclin D1*, *Myc*, and *adenovirus early region 1A-associated protein 300* in lung homogenate of murine OVA-induced AAI. Our finding of a significant increase in *Wnt5a* expression in mice with AAI compared with healthy controls complements previous studies and suggests that, in addition to canonical signaling, β -catenin-independent, noncanonical WNT signaling is also involved in asthma pathogenesis. In humans, *WNT5a* expression has been associated with a T helper type 2 signature in airways (8) and is increased in ASM cells of patients with mild to moderate asthma. Furthermore, an siRNA-mediated knockdown of *WNT5a* reduced the deposition of extracellular matrix by ASM cells (13). Thus, noncanonical WNT signaling via *WNT5a* might be implicated in the early development of airway remodeling in asthma. Whether this is also (indirectly) regulated by *miR-142-3p* needs to be assessed in future studies.



In summary, our extensive qRT-PCR-based screening approach revealed alterations in several factors of canonical and noncanonical WNT signaling in early onset versus late onset severe asthma. Because here we could only assess a small number of patients per group, the results need to be validated in larger studies in the future, which should be combined with an unbiased transcriptomics approach to characterize in-depth distinct molecular endotypes and pave the way for precision medicine approaches.

Finally, our data suggest an involvement of *miR-142-3p* in regulating the balance between proliferation and differentiation of ASM cells in the pathogenesis of asthma. This study complements previous work on this topic, and we propose *miR-142-3p* as an interesting molecular target to prevent early ASM hyperproliferation in asthma.

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DISCLOSURES

S. Bartel has received grants for research from Bencard Allergie GmbH and is a member of the scientific advisory board, none of which is related to the content of this article.

AUTHOR CONTRIBUTIONS

S.K.-E., F.L.M.R., and S. Bellusci conceived and designed research; S. Bartel, G.C., and F.A. performed experiments; S. Bartel, G.C., and F.A. analyzed data; S. Bartel, G.C., F.A., S.K.-E., and F.L.M.R. interpreted results of experiments; S. Bartel and G.C. prepared figures; S. Bartel drafted manu-

Fig. 3. Quantitative (q)RT-PCR for 78 WNT-associated genes in bronchial biopsies from patients with early ($n = 5$) and late onset ($n = 4$) severe asthma. Mean $\pm$ SD: * $P < 0.05$. WNT, Wingless/Integrase I.

Stratification in early or late onset of severe asthma enabled us to identify different “molecular disease endotypes.” We observed higher *miR-142-3p* levels in patients with a late onset. Because of ethical limitations, we could not compare the

script; S. Bartel, F.L.M.R., and S. Bellusci edited and revised manuscript; G.C., F.A., S.K.-E., F.L.M.R., and S. Bellusci approved final version of manuscript.

REFERENCES

1. Baarsma HA, Königshoff M. 'WNT-er is coming': WNT signalling in chronic lung diseases. *Thorax* 72: 746–759, 2017. doi:10.1136/thoraxjnl-2016-209753.
2. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 347: 911–920, 2002. doi:10.1056/NEJMra020100.
3. Barreto-Luis A, Corrales A, Acosta-Herrera M, Gonzalez-Colino C, Cumplido J, Martinez-Tadeo J, Carracedo A, Villar J, Carrillo T, Pino-Yanes M, Flores C. A pathway-based association study reveals variants from Wnt signalling genes contributing to asthma susceptibility. *Clin Exp Allergy* 47: 618–626, 2017. doi:10.1111/cea.12883.
4. Bartel S, Schulz N, Alessandrini F, Schamberger AC, Pagel P, Theis FJ, Milger K, Noessner E, Stick SM, Kicic A, Eickelberg O, Freishtat RJ, Krauss-Etschmann S. Pulmonary microRNA profiles identify involvement of Creb1 and Sec14l3 in bronchial epithelial changes in allergic asthma. *Sci Rep* 7: 46026, 2017. doi:10.1038/srep46026.
5. Carraro G, El-Hashash A, Guidolin D, Tiozzo C, Turcatel G, Young BM, De Langhe SP, Bellusci S, Shi W, Parnigotto PP, Warburton D. miR-17 family of microRNAs controls FGF10-mediated embryonic lung epithelial branching morphogenesis through MAPK14 and STAT3 regulation of E-Cadherin distribution. *Dev Biol* 333: 238–250, 2009. doi:10.1016/j.ydbio.2009.06.020.
6. Carraro G, Shrestha A, Rostkovius J, Contreras A, Chao C-M, El Agha E, Mackenzie B, Dilai S, Guidolin D, Taketo MM, Günther A, Kumar ME, Seeger W, De Langhe S, Barreto G, Bellusci S. miR-142-3p balances proliferation and differentiation of mesenchymal cells during lung development. *Development* 141: 1272–1281, 2014. doi:10.1242/dev.105908.
7. Chen CY, Chen ST, Fuh CS, Juan HF, Huang HC. Coregulation of transcription factors and microRNAs in human transcriptional regulatory network. *BMC Bioinformatics* 12, Suppl 1: S41, 2011. doi:10.1186/1471-2105-12-S1-S41.
8. Choy DF, Modrek B, Abbas AR, Kummerfeld S, Clark HF, Wu LC, Fedorowicz G, Modrusan Z, Fahy JV, Woodruff PG, Arron JR. Gene expression patterns of Th2 inflammation and intercellular communication in asthmatic airways. *J Immunol* 186: 1861–1869, 2011. doi:10.4049/jimmunol.1002568.
9. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, Adcock IM, Bateman ED, Bel EH, Bleecker ER, Boulet L-P, Brightling C, Chaney P, Dahlen S-E, Djukanovic R, Frey U, Gaga M, Gibson P, Hamid Q, Jajour NN, Mauad T, Sorkness RL, Teague WG. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 43: 343–373, 2014 [Erratum in *Eur Respir J* 43:1216, 2014] doi:10.1183/09031936.00202013.
10. Fehrenbach H, Wagner C, Wegmann M. Airway remodeling in asthma: what really matters. *Cell Tissue Res* 367: 551–569, 2017. doi:10.1007/s00441-016-2566-8.
11. Goswami S, Angkasekwinai P, Shan M, Greenlee KJ, Barranco WT, Polikepahad S, Seryshev A, Song LZ, Redding D, Singh B, Sur S, Woodruff P, Dong C, Corry DB, Kheradmand F. Divergent functions for airway epithelial matrix metalloproteinase 7 and retinoic acid in experimental asthma. *Nat Immunol* 10: 496–503, 2009. doi:10.1038/ni.1719.
12. Isobe T, Hisamori S, Hogan DJ, Zabala M, Hendrickson DG, Dalerba P, Cai S, Scheeren F, Kuo AH, Sikandar SS, Lam JS, Qian D, Dirbas FM, Somlo G, Lao K, Brown PO, Clarke MF, Shimono Y. miR-142 regulates the tumorigenicity of human breast cancer stem cells through the canonical WNT signaling pathway. *eLife* 3: e01977, 2014. doi:10.7554/eLife.01977.
13. Kumawat K, Menzen MH, Bos IS, Baarsma HA, Borger P, Roth M, Tamm M, Halayko AJ, Simoons M, Prins A, Postma DS, Schmidt M, Gosens R. Noncanonical WNT-5A signaling regulates TGF- β -induced extracellular matrix production by airway smooth muscle cells. *FASEB J* 27: 1631–1643, 2013. doi:10.1096/fj.12-217539.
14. Maes T, Cobos FA, Schleif F, Sorbello V, Henket M, De Preter K, Bracke KR, Conicx G, Mesnil C, Vandesompele J, Lahousse L, Bureau F, Mestdagh P, Joos GF, Ricciardolo FL, Brusselle GG, Louis R. Asthma inflammatory phenotypes show differential microRNA expression in sputum. *J Allergy Clin Immunol* 137: 1433–1446, 2016. doi:10.1016/j.jaci.2016.02.018.
15. Perry MM, Adcock IM, Chung KF. Role of microRNAs in allergic asthma: present and future. *Curr Opin Allergy Clin Immunol* 15: 156–162, 2015. doi:10.1097/ACI.0000000000000147.
16. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29: e45, 2001. doi:10.1093/nar/29.9.e45.
17. Ricciardolo FL, Sabatini F, Sorbello V, Benedetto S, Defilippi I, Petecchia L, Usai C, Gnemmi I, Balbi B, De Rose V, Ten Hacken NH, Postma DS, Timens W, Di Stefano A. Expression of vascular remodeling markers in relation to bradykinin receptors in asthma and COPD. *Thorax* 68: 803–811, 2013. doi:10.1136/thoraxjnl-2012-202741.
18. Sabatini F, Luppi F, Petecchia L, Stefano AD, Longo AM, Eva A, Vanni C, Hiemstra PS, Sterk PJ, Sorbello V, Fabbri LM, Rossi GA, Ricciardolo FL. Bradykinin-induced asthmatic fibroblast/myofibroblast activities via bradykinin B2 receptor and different MAPK pathways. *Eur J Pharmacol* 710: 100–109, 2013. doi:10.1016/j.ejphar.2013.03.048.
19. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, Denney L, Bossley CJ, Oates T, Walker SA, Bush A, Lloyd CM. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. *J Allergy Clin Immunol* 132: 676–685.e13, 2013. doi:10.1016/j.jaci.2013.04.012.
20. Sessa R, Hata A. Role of microRNAs in lung development and pulmonary diseases. *Pulm Circ* 3: 315–328, 2013. doi:10.4103/2045-8932.114758.
21. Sharma S, Tantisira K, Carey V, Murphy AJ, Lasky-Su J, Celedón JC, Lazarus R, Klanderman B, Rogers A, Soto-Quirós M, Avila L, Mariani T, Gaedigk R, Leeder S, Torday J, Warburton D, Raby B, Weiss ST. A role for Wnt signaling genes in the pathogenesis of impaired lung function in asthma. *Am J Respir Crit Care Med* 181: 328–336, 2010. doi:10.1164/rccm.200907-1009OC.
22. Shrestha A, Mukhametshina RT, Taghizadeh S, Vázquez-Pacheco E, Cabrera-Fuentes H, Rizvanov A, Mari B, Carraro G, Bellusci S. *MicroRNA-142* is a multifaceted regulator in organogenesis, homeostasis, and disease. *Dev Dyn* 246: 285–290, 2017. doi:10.1002/dvdy.24477.
23. Wang SH, Xu F, Dang HX, Yang L. Genetic variations in the Wnt signaling pathway affect lung function in asthma patients. *Genet Mol Res* 12: 1829–1833, 2013. doi:10.4238/2013.January.4.1.
24. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 18: 716–725, 2012. doi:10.1038/nm.2678.
25. World Health Organization. *Asthma*. (Online). <http://www.who.int/respiratory/asthma/en/> [accessed on 15 January 2018].