Toxicology and Applied Pharmacology 301 (2016) 61–70

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Alteration of canonical and non-canonical WNT-signaling by crystalline silica in human lung epithelial cells

Timothy N. Perkins [a], Mieke A. Dentener [a], Frank R. Stassen [b], Gernot G. Rohde [a], Brooke T. Mossman [c], Emiel F.M. Wouters [a], Niki L. Reynaert [a,*]

[a] Department of Respiratory Medicine, Maastricht University Medical Centre +, Maastricht University Maastricht, The Netherlands
[b] Department of Medical Microbiology, Maastricht University Medical Centre +, Maastricht University Maastricht, The Netherlands
[c] Department of Pathology, University of Vermont College of Medicine, Burlington, VT, USA

ARTICLE INFO

Article history:
Received 23 October 2015
Revised 5 April 2016
Accepted 6 April 2016
Available online 16 April 2016

Keywords:
Silica
WNT
Pneumoconiosis
Fibrosis
Pathway-analysis
Epithelium

ABSTRACT

Growth and development of the mature lung is a complex process orchestrated by a number of intricate developmental signaling pathways. Wingless-type MMTV-integration site (WNT) signaling plays critical roles in controlling branching morphogenesis cell differentiation, and formation of the conducting and respiratory airways. In addition, WNT pathways are often re-activated in mature lungs during repair and regeneration. WNT- signaling has been elucidated as a crucial contributor to the development of idiopathic pulmonary fibrosis as well as other hyper-proliferative lung diseases. Silicosis, a detrimental occupational lung disease caused by excessive inhalation of crystalline silica dust, is hallmarked by repeated cycles of damaging inflammation, epithelial hyperplasia, and formation of dense, hyalinized nodules of whorled collagen. However, mechanisms of epithelial cell hyperplasia and matrix deposition are not well understood, as most research efforts have focused on the pronounced inflammatory response. Microarray data from our previous studies has revealed a number of WNT-signaling and WNT-target genes altered by crystalline silica in human lung epithelial cells. In the present study, we utilize pathway analysis to designate connections between genes altered by silica in WNT-signaling networks. Furthermore, we confirm microarray findings by QRT-PCR and demonstrate both activation of canonical (β-catenin) and down-regulation of non-canonical (WNT5A) signaling in immortalized (BEAS-2B) and primary (PBEC) human bronchial epithelial cells. These findings suggest that WNT-signaling and cross-talk with other pathways (e.g. Notch), may contribute to proliferative, fibrogenic and inflammatory responses to silica in lung epithelial cells.

Abbreviations: ACTA2, Actin, alpha, smooth muscle; ANGPTL4, Angiopoietin-like 4; ANOVA, Analysis of variance; AXIN, Axis inhibition protein; BMP, Bone morphogenic protein; BMPR1B, BMP receptor 1B; CACNA2D3, Calcium channel, voltage-dependent, alpha 2/delta subunit 3; CCND, Cyclin-D; CD44, CD44 molecule; CDKN, Cyclin-dependent kinase inhibitor; CLDN1, Claudin-1; CM, Conditioned medium; CON, Control; COPD, Chronic obstructive pulmonary disease; COX2, Cyclooxygenase-2; CREB, Cyclic-AMP response element binding protein; CREB3L1, CREB-3-like 1; CTGF, Connective tissue growth factor; DAAM2, Dishevelled associated activator of morphogenesis 2; DAB2, Disabled homolog 2, mitogen-responsive phosphatase; DKK, Dickkopf homolog; EFNB2, Ephrin B2; EGR, Early growth response; FGF, Fibroblast growth factor; FOSL1/FRA1, Fos-like antigen; FZD, Frizzled; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; GJA1, Gap junction protein, alpha 1; GTP, Guanosine triphosphate; HES, Hairy enhancer; HEY, Hairy/enhancer-of-split related with YRPW motif 1; HSPA12A, Heat shock 70 kDa protein 12A; IFNB, Interferon beta; IL, Interleukin; IPA, Ingenuity pathway analysis; IPF, Idiopathic pulmonary fibrosis; JAG1, Jagged 1; JNK, c-jun N-terminal kinase; JUN, c-jun proto-oncogene; LD_{50}, Lethal dose < 50; LEF, Lymphoid enhancer; LRP, Low-density lipoprotein receptor; LRP5L, LRP-related protein-like 5; MDM2, MDM2 oncogene, E3 ubiquitin protein ligase; MMP, Matrix metallopeptidase; MUMC, Maastricht University Medical Centre; MYC, c-myc proto-oncogene; NFAT, Nuclear factor of activated T-cells; NFKB, Nuclear factor kappa B; NHBE, Normal human bronchial epithelial cells (Lonza); NOTCH, Notch ligand; NRCAM, Neuronal cell adhesion molecule; NSCLC, Non-small cell lung cancer; PBEC, Primary bronchial epithelial cells; PLAUR, Plasminogen activator urokinase receptor; PLCL2, Phospholipase C-like 2; PLUC, Primary Lung Culture Facility; PML, Pro-myelocytic leukemia; PPAP2B, Phosphatidic acid phosphatase type 2B; QRT-PCR, Quantitative reverse transcriptase polymerase chain reaction; RELB, v-Rel avian reticuloendotheliosis viral oncogene homolog B; RPL13A, Ribosomal protein L13A; SEM, Standard error of the mean; SFRP, Secreted frizzled-related protein; SIL, Silica; siRNA, Small-interfering RNA; SOX, SRY (sex-determining region) box; TCF, T-cell specific factor; TGFBR3, Transforming growth factor beta receptor 3; TLE3, Transducin-like enhancer of split 3; TP53, Tumor protein 53; TP53BP2, TP53 binding protein 2; TP53I3, TP53 inducible protein 3; UBD, Ubiquitin D; VEGFA, Vascular endothelial growth factor A; WCL, Whole cell lysate; WIF, WNT inhibitory factor; WISP2, WNT inducible protein 2; WNT, Wingless-type MMTV-integration site.

* Corresponding author at: Department of Respiratory Medicine, Maastricht University Medical Centre, PO Box 616, 6200MD Maastricht, The Netherlands.
E-mail address: n.reynaert@maastrichtuniversity.nl (N.L. Reynaert).

http://dx.doi.org/10.1016/j.taap.2016.04.003
0041-008X/

1. Introduction

Development of the mature lung is a complex process involving branching morphogenesis and growth of the conducting airways followed by respiratory airway formation and finally development of the alveolar airspaces. These processes are orchestrated by dynamic cell differentiation patterns and intricate cross-talk between mesenchymal and epithelial cell types (Chang et al., 2013; Desai et al., 2014; Rockich et al., 2013; Varner and Nelson, 2014; Herriges and Morrisey, 2014). Several signaling pathways including fibroblast growth factor (FGF), wingless type MMTV integration site (WNT), are implicated in coordination of lung development (Hines and Sun, 2014). Furthermore, such signaling pathways are vital to homeostasis of the mature lung, whereby they mediate self-renewal and regeneration of the airways and alveoli upon injury and cell turnover (Hogan et al., 2014). However, in hyper-proliferative diseases, developmental pathways may become disrupted during regeneration and repair leading to excessive cell turnover, compensatory proliferation, and imbalanced matrix dissolution and deposition (Herriges and Morrisey, 2014; Hogan et al., 2014; Beers and Morrisey, 2011).

WNT-signaling is highly active and strictly regulated in developmental processes (Volckaert and De Langhe, 2015; Nusse, 2012). On the other hand, WNT-signaling has been implicated as a driving force in the development and progression of chronic lung diseases such as emphysema and chronic obstructive pulmonary disease (COPD) (Lam et al., 2011; Pain et al., 2014; Heijink et al., 2013; Chilosi et al., 2003, 2012). WNT is a family of 19 secreted glycoproteins, which bind to heterodimeric receptor complexes of frizzled receptors (FZD) and low-density lipo-protein receptors (LRP) 5 and 6, initiating down-stream signaling, to promote cell proliferation, migration and differentiation (MacDonald et al., 2009; Willert and Nusse, 2012). WNT-signaling is classified into 3 main pathways. Canonical WNT/β-catenin, which promotes cell proliferation, and two non-canonical pathways, WNT/c-jun N-terminal kinase (JNK), which controls cell polarity and cytoskeletal rearrangement, and the WNT/Ca^{+} pathway which controls cell migration and adhesion (MacDonald et al., 2009; Komiya and Habas, 2008; Gordon and Nusse, 2006).

WNT-signaling is regulated by a number of secreted inhibitors, including secreted frizzled-related proteins (sFRPs) and WNT-inhibitory factors (WIFs) which inhibit signaling by direct contact with WNT-ligands, whereas the Dickkopf WNT-signaling pathway inhibitors (DKKs) interact with receptor complexes (Kawano and Kypta, 2003). Moreover, WNT-signaling is also regulated intracellularly by the scaffolding proteins, Axis Inhibition proteins (AXINs) by promoting degradation of β-catenin (Behrens et al., 1998; Kikuchi, 1999; Lee et al., 2003; Li et al., 2012). In addition, it has been demonstrated that different WNT pathways regulate each other via crosstalk, e.g. non-canonical WNT-signaling (WNT5A) can both activate and repress canonical WNT-signaling (Yao et al., 2014).

Reactivation of WNT-signaling in mature lungs has been viewed as a key-contributing factor in the initiation and development of pulmonary diseases. In lung cancers, over-activation of WNT-signaling is evidenced by increased expression of WNT ligands, as well as decreased expression of WNT-inhibitors by epigenetic-silencing (Yao et al., 2014; Nakashima et al., 2010; Tao et al., 2015; Zhang et al., 2010). Recently, WNT signaling has also been elucidated as a key contributor in the development of idiopathic pulmonary fibrosis (IPF) (Chilosi et al., 2003). Studies have revealed increased expression of WNT ligands as well as target genes in IPF lungs (Konigshoff et al., 2008) with prominent activation of WNT-signaling components in the lung epithelium, especially during the regenerative stages of experimental pulmonary fibrosis (Konigshoff et al., 2008; Meuten et al., 2012). Current understandings suggest autocrine and paracrine WNT signaling leads to epithelial hyperplasia, and matrix remodeling in lung fibrosis (Konigshoff and Eickelberg, 2010).

In occupations such as mining, sandblasting and ceramics, long term exposure to respirable crystalline silica is associated with the development of pulmonary fibrosis (silicosis), as well as lung cancers, particularly in smokers (Mossman and Churg, 1998). Development of silicosis is hallmarked by deposition of inhaled particles, prolonged cycles of massive inflammation, epithelial hyperplasia, fibroblast proliferation, and deposition of extracellular matrix components (Perkins et al., 2014a). This results in the development of distinct silicotic nodules, which can grow, coalesce, and eventually cause progressive massive pulmonary fibrosis (Castranova and Vallyathan, 2000). However, mechanisms of epithelial cell hyperplasia and matrix deposition are not well understood, as the majority of research has focused on the intense and prolonged inflammatory response in silicosis.

Microarray data from our previous studies of gene expression profiling in lung epithelial cells exposed to crystalline silica suggests WNT-signaling is modulated by silica (Perkins et al., 2012, 2014b). As seen in multiple fibrotic disorders, consequent autocrine and paracrine signaling likely contributes to epithelial regeneration and recruitment of fibroblasts leading to matrix breakdown and deposition in silicosis (Perkins et al., 2014a). The goals of this study were to utilize pathway analysis to draw connections between potential WNT-signaling networks, confirm microarray findings, and demonstrate whether canonical, and/or non-canonical WNT-signaling pathways are induced by crystalline silica in human lung epithelial cells.

2. Materials and methods

2.1. Culture and silica exposures in BEAS-2B and NHBE cells

Culturing of both BEAS-2B and Normal human bronchial epithelial cells (NHBE) commercially available (Lonza, Clonetics) was performed as previously described (Perkins et al., 2012). Cells were exposed to Cristobalite silica (King of Prussia, PA), which was previously characterized (Perkins et al., 2012) with a mean diameter of 2.16 ± 2.00 μm. Cells were exposed to non-lethal doses (less than LD_{50}) for 24 h, as previously described (Perkins et al., 2012). In brief, silica dust was dispersed in sterile saline solution at a concentration of 1 mg/ml. Prior to exposure, silica samples were sonicated for 15 min in a water bath sonicator and trichurated through a 25G needle prior to administration.

2.2. Isolation and culture of primary bronchial epithelial cells (pBECs)

Lung tissue used for the isolation of PBECs was obtained from the Maastricht Pathology Tissue Collection (MPTC). Collection, storage and use of tissue and patient data were performed in agreement with the "Code for Proper Secondary Use of Human Tissue in the Netherlands" (http://www.fmwv.nl). The scientific board of the MPTC approved the use of materials for this study under MPTC2010-019. PBECs were isolated from resected lung tissue of 3 patients without known history of chronic lung diseases who underwent surgery for solitary pulmonary nodules. Isolation, culture and characterization of cells was performed by the Primary Lung Culture (PLUC) facility at the Maastricht University Medical Centre (MUMC) as previously described (van Wetering et al., 2000).

Briefly, cells were detached from bronchus rings by protease XIV digestion (Sigma, St. Louis, MO, USA), washed and cultured in defined keratinocyte serum-free medium (KSFM, Gibco, life technologies, NY, USA) containing 1 μM isoproterenol (Sigma, St. Louis, MO, USA), penicillin/streptomycin (GE Healthcare Life Sciences, Eindhoven, The Netherlands) and MycoZap PlusPR (Lonza, Verviers, Belgium). Culture dishes were pre-coated using 30 μg/ml PureCol (Advanced Biomatrix, San Diego, CA, USA), 10 μg/ml human fibronectin (Becton Dickinson, Franklink Lakes, NJ, USA) and 10 μg/ml BSA (Sigma, St. Louis, MO, USA). When cell layers reached approximately 80% confluency, cells were trypsinized and cryopreserved until further use. Epithelial