INTRODUCTION
The use of more reliable and relevant human in vitro models as well as Systems Biology-based approaches to investigate the impact of toxins such as cigarette smoke (CS) is in line with the recent shift of toxicological assessment strategy from finding alternatives for animal testing and to systems level questions in an integrative way. We recently published a comparison study which demonstrated that organotypic cultures (OC) of human bronchial epithelial cells (HBE) exposed to CS at atmospheric conditions (Fig. 2) can recapitulate the biology observed in the bronchial epithelium of smokers (1). In this study, data from large scale mRNA, miRNA, protein analysis, and from immunohistochemical analyses were captured at different post-exposure time points (0.5h, 2h, 6h, 4h, 24h). Here, we will give the results of the overall approach (4) on the perturbations of pathways related to cell proliferation (Fig. 3 and 4), focusing on two exposure periods (14 and 28 min) and on all post-exposure time points. We will also substantiate this analysis with immunohistochemical observations (Fig. 5) and suggest dose-dependent reparative mechanisms above the cells.

MATERIALS & METHODS

RESULTS

CONCLUSIONS

REFERENCES

4. www.mousepathway.com

With this study, we described the application of a system biology-based approach to get a comprehensive view on the impact of whole CS exposure to human organotypic bronchial epithelial cell culture combining molecular endpoints with histological measurements. Investigating different doses and post-exposure periods up to 4h, we showed a recovery of the cellular response at lower CS concentrations, whereas at higher concentrations a sustained perturbation of cellular networks was visible.

By applying Reverse Causal Reasoning (RCR) as a data analysis strategy (e.g. Cell Proliferation, Cellular Stress, Inflammatory Response (5)), we investigated the different cellular pathways perturbed by CS exposure which were related to cellular stress response, the inflammation and the proliferation/differentiation mechanisms. Results from this new approach were compared to the current systems biology models (e.g. transcriptional activity of HIF, Wnt/beta-catenin, MAPK activity, etc.). We found that the CS exposure resulted in a decrease of the pathway activity, which was significant for both high and low concentrations. The proportion diminished significantly 24h after the exposure, compared to sham and a higher decrease observed for the higher dose (Fig. 5B). The number of ciliated cells which are the other cell type directly in contact with the smoke is on the other hand unchanged compared to sham (Fig. 5A). Interestingly, the proportion of basal cells (Fig. 5C) decreases inversely in the same proportion and at the same time that the non ciliated cells (Fig. 5D). This observation could suggest a regeneration process where the damaged cells from the apical side are replaced by non ciliated cells that will later on differentiate and compensate for the loss of goblet cells (Fig. 5B). In parallel to the increase of non ciliated cells proportions, the activation of HIF for cell cycle inducers or related to the Growth Factor Network (Fig. 4) was observed. The whole scenario of the tissue integrity regeneration implies processes such as spreading, migration, differentiation, and proliferation that are involved in the epithelial wound repair in lung (5), although one important cell type, the fibroblasts were missing in the AIR-100 tissue. Based on the HIF analysis it seems that EGR-related processes are involved in the proliferation signaling. The EGFR pathway is activated in many human lung adenocarcinoma either CS-dependently via K-Ras activation or CS-independently by EGR amplification (6). Goblet cells might also be present in the CS-exposed AIR-100 tissue. Collectively our results suggest a dose-dependent recovery of the damaged tissue involving probably mechanisms known from epithelial repair in human lung. To further characterize the late activity of the AIR-100 tissue in our experimental set-up, longer post-exposure period would be worth to investigate.