ÉCOLE DOCTORALE UNIVERSITÉ PARIS-EST
Sciences de la Vie et de la Santé

Campagne de recrutement 2020

Titre du projet de Thèse
Study of the regulation of the expression of the *NKX2-1* gene and its involvement in abnormalities in surfactant metabolism

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Contexte scientifique du projet et objectifs (0,5 page maximum)

Arial 11, interligne simple

Inherited abnormalities of surfactant metabolism account for 10-15% of the causes of respiratory syndromes associated with alveolar-interstitial lung disease in children. Alveoli, which are affected in these diseases, are the functional units of the lung and in charge of various functions such as gas exchange and barrier function. The alveolar epithelium consists of three cell types: alveolar epithelial cells type 1 (AEC1) and type 2 (AEC2) as well as macrophages. AEC2 cells synthesize and secrete the surfactant that lines the alveolar surface to reduce the surface tension and allows expansion of the lung during inspiration. Heretofore, mutations have been described mainly in four genes that encode proteins expressed by AEC2 cells and involved in surfactant production: *SFTPB*, *SFTPC*, *ABCA3*, and *NKX2-1*. In the current project, our interest is focused on the transcription factor NKX2-1 which is a master regulator of normal lung development and alveolar cell lineage commitment. *NKX2-1* gene defects occur either as sporadic cases or as familial cases inherited in an autosomal dominant way. The severity of symptoms varies considerably, even in families with the same disease-causing mutation highlighting that other factors may be involved (Nattes 2017). In this framework, to get insight into the mechanistic of mutation expression (including protein defects and absence of the protein), we have developed functional studies. Nowadays, 3D organoids derived from stem cell differentiation have emerged as a new tool to reproduce and study respiratory diseases in a culture system. In this framework, we start the development of an iPSC-derived alveolar epithelium which will allow us to properly analyze both *NKX2-1* gene mutations and regulation of the gene.

The goal of the PhD project is to determine if the expression control of the *NKX2-1* gene depends on long non-coding RNAs and evaluate if this control could be a key to develop some drug treatments. The first objective will be to develop an iPSC-derived alveolar epithelium cultured in 3D or air-liquid conditions. The second objective will be to decipher if and how the two long non-coding RNAs (lncRNA) flanking the *NKX2-1* gene affect the control of its expression. The third objective will be to evaluate whether some actors involved in *NKX2-1* gene regulation through the lncRNA could be useful as drugs to overcome the *NKX2-1* defect.

The project will require the use of several approaches and techniques including culture and differentiation of iPSC, flow cytometry, imaging analyses, genome editing with CRISPR-Cas9 and -dCas9 tools, RNA analyses (qRT-PCR or RNAseq), and regular molecular biology approaches.

Compétences requises du candidat (5 lignes maximum)

Arial 11, interligne simple

The candidate must demonstrate motivation, determination, and perseverance. He/she must also be rigorous in organizing his/her experiments, and then for analyzing and presenting his/her results. He/she must know how to organize his/her work independently, including the management of the bibliography while interacting positively with team members. Knowledge and practice in iPSC cell culture would be a real asset. He/she must have acquired skills in the fields of cell and molecular biology. Finally, a candidate who is stimulated as much by the realization of the experimental work as by the more theoretical aspect of his/her project would be highly appreciated.

Publications de l'équipe d'accueil de ces 5 dernières années en lien avec le projet (5 publications maximum)

1. Gros M, Aissat A, Perez-Martin S, Abou Taam R, Funalot B, Fanen P, Epaud R, de Becdelievre A. Interstitial lung disease reveals 48,XXYY syndrome in a child. Acta Paediatr. 2020 May;109(5):1060-1061.
2. Degrugillier F, Simon S, Aissat A, Remus N, Mekki C, Decrouy X, Hatton A, Hinzpeter A, Hoffmann B, Sermet-Gaudelus I, Callebaut I, Fanen P, Prulière-Escabasse V. Unsolved severe chronic rhinosinusitis elucidated by extensive CFTR genotyping. Clin Case Rep. 2019 Sep 27;7(11):2128-2134.

3. Nattes E, Lejeune S, Carsin A, Borie R, Gibertini I, Balinotti J, Nathan N, Marchand-Adam S, Thumerelle C, Fauroux B, Bosdure E, Houdouin V, Delestrain C, Louha M, Couderc R, De Becdelievre A, Fanen P, Funalot B, Crestani B, Deschildre A, Dubus JC, Epaud R. Heterogeneity of lung disease associated with NK2 homeobox 1 mutations. Respir Med. 2017 Aug;129:16-23.
4. Delestrain C, Simon S, Aissat A, Medina R, Decrouy X, Nattes E, Tarze A, Costes B, Fanen P, Epaud R. Deciphering the mechanism of Q145H SFTPC mutation unmasks a splicing defect and explains the severity of the phenotype. Eur J Hum Genet. 2017 Jun;25(6):779-782.
5. Epaud R, Delestrain C, Louha M, Simon S, Fanen P, Tazi A. Combined pulmonary fibrosis and emphysema syndrome associated with ABCA3 mutations. Eur Respir J. 2014 Feb;43(2):638-41.