Journal of Infectious Diseases & Therapy
ISSN: 2332-0877

Huang, J Infect Dis Ther 2021, 9:S5

Review Article Open Access

# The Ontogeny of Red Soma Cells

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**Received date:** March 31, 2021; **Accepted date:** April 16, 2021; **Published date:** April 23, 2021



## Abstract

We reported recently our discovery of an important population of red cells in the lungs, distinct from the Red blood cells. They are permanent resident pulmonary cells located in the connective tissues of the lungs, with multiple differentiation potentials and diverse important functions. We named this population of cells, Red Soma Cells. In this article, we try to map out the ontogeny of the Red Soma Cells during mouse lung development. First, based on our knowledge on RSC cells in adult human lungs, we established an "Identikit" of RSC cells: a disk-like shape and pink-colored after Eosin/Hematoxylin staining on histological sections. Then, with this "Identikit" in mind, we searched for RSC-like cells in the literature on mouse lung development. We found at least 7 lines of evidence, which permitted us to construct a Mind Map on the ontogeny of Red Soma Cells during mouse lung development. This Mind Map allowed us to explain a large amount of experimental data obtained in several different fields, with different analytical tools: Anatomy/Whole lungs, Histology/Lung sections and H/E staining, Genetics/transgenic mice, Gene expression analysis/Whole genome transcriptomes, Developmental Biology/septation and Ontogeny of lung macrophages, etc. Finally, we reached the following conclusion: the mouse Red Soma Cells, probably originated from fetal liver, constitute a unique cell population of Hematopoietic Stem Cells with important roles in mouse lung development.

**Keywords:** Ontogeny; Red soma cells; Shh; Foxf1; Lung; Macrophages

## Introduction

We reported recently our discovery of an important population of red cells in the lungs, distinct from the Red blood cells. They are permanent resident pulmonary cells located in the connective tissues of the lungs, with multiple differentiation potentials and diverse important functions. We named this population of cells, Red Soma Cells [1].

However, one important question remained to be answered: What is the origin of those RSC cells? By searching the literature on mouse lung development, we found 7 lines of evidence that allowed us to map out the ontogeny of Red Soma Cells during the development of the mouse lungs (Figure 1). All the references cited in this article are available and accessible online.

Needless to say, cells, especially the stem cells, have a great capacity to adapt to various environments and to display a variety of behaviors. However, despite of their stem cell features, we have never succeeded in amplifying the mature alveolar macrophages in culture, no matter what kind of stimuli that we had added. If we understand correctly, the mature macrophages implied by Sieweke and Allen [11] had been genetically modified: They harbored a combined deficiency for the transcription factors MafB and c-Maf [13]. In light of our discovery of RSC cells, it would be premature to rule out the possible involvement of progenitor cells in the maintenance of macrophage populations in the lungs.

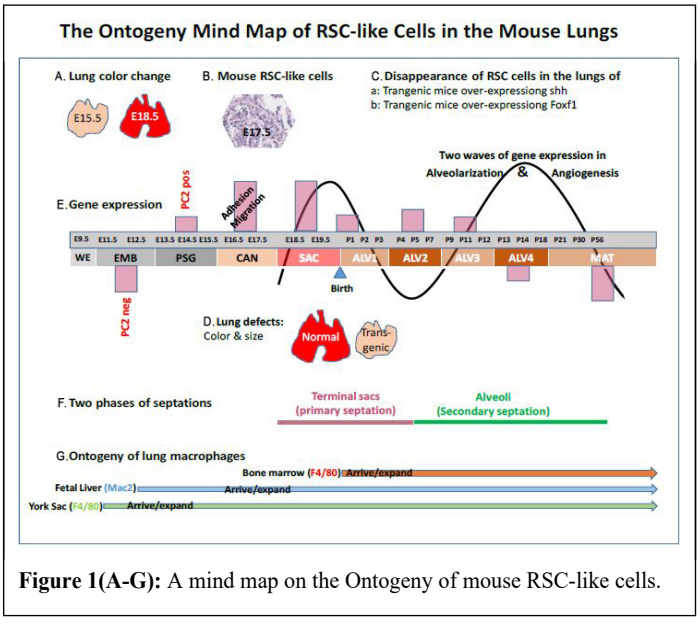


**Figure 1(A-G):** A mind map on the Ontogeny of mouse RSC-like cells.

The color change of the developing mouse lungs from brownish at E15.5 to red at E18.5 (Figures 1A-1G) [2], might be a sign of the arrival of RSC-like cells in the developing mouse lungs.

Indeed, RSC-like cells could be observed on the E17.5/E18.5 mouse lung sections stained with Eosin/Hematoxylin (Figure 1E) and (Figures 2A and 2B). control. These cells disappeared in the lung of the transgenic mouse over-expressing sonic hedgehog (Shh) (Figure 1E). The same phenomenon was also observed in the lungs of transgenic mice conditionally expressing Foxf1 in endothelial and hematopoietic lineages (Figures 2A and 2B).

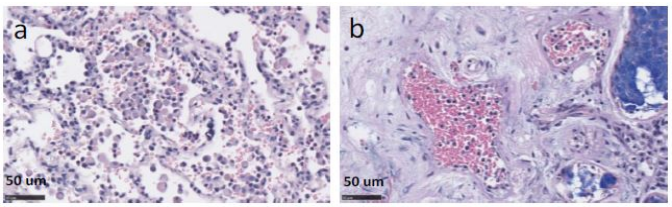


**Figure 2:** The morphology of RSC cells in the lungs of CF donors. A) CF lung parenchyma, showing the presence of RSC cells in the deformed alveolar spaces; B) Clusters of RSC cells on a section of bronchus of CF donors, after Eosin and Hematoxylin staining.

Probably, the absence of RSC-like cells might have caused the lethal lung hypoplasia and vascular defects, and the loss of red color.

All these events are correlated with the change of gene expression profiles: there was a great increase of expression of genes involved in cell Adhesion/Migration between E16.5 and E19.5 (Figure 1D). The arrival of RSC-like might have initiated two waves of Alveolarization and Angiogenesis from E18.5 onwards (Figure 1F).

Alveolarization and Angiogenesis are the two sides of the same coin, namely the formation of septum during the mouse lung development. Alveolarization and Angiogenesis are intrinsically linked and coordinated during septum formation. That may be the reason why Alveolarization and Angiogenesis showed the same gene expression profiles.

Analysis of the data on the ontogeny of lung macrophages suggested that RSC-like cells could be derived from the mouse fetal liver and function as the progenitor cells of alveolar.

## The Red Color

We believe that RSC cells that we identified in the adult lungs must be of embryonic origin. In our previous article on the discovery of RSC cells, we used the red color as a marker to follow the RSC cells in the adult lungs. This strategy should also be applicable with regard to the developing mouse lungs. In other words, it may be possible to trace these cells during early lung development just by examining the color of the lungs. Searching the literature on mouse lung development, we did find some images showing the color change of the developing mouse lungs: In Figure 1G of an article published by Galambos et al. (Figure 1G), one could clearly see that the mouse lungs changed the color from brownish at E15.5 to reddish at E18.5 [2].

The same question was raised again: What made the developing mouse lungs turned red at E18.5? Red Blood Cells or Red Soma Cells? There were several lines of evidence in favor of the Red Soma Cells. The first argument would be the timing of this color change: E18.5 seems to be the starting point of Alveolization and Angiogenesis of the mouse lung. By profiling the genome wide gene expression, Beauchemin et al. found at least two waves of episodic transcriptional activity of genes related to pulmonary vascularization and Angiogenesis, between E18.5 and P56 (Figure 1F) [3]. Based on this chronology, two conclusions were

- What inescapable:made the developing mouse lungs look red at

E18.5 was not red blood cells, because the blood vessels, especially the capillary bed, had not yet formed.

- The color change and the angiogenesis were closely related. In other words, these red cells must somehow participate actively in angiogenesis in the developing mouse lungs.

## Morphology, Histology and Genetics

To look for more evidence, we used our knowledge of RSC cells that we identified in the lungs of adult rat and human beings. In fact, the RSC cells have a particular morphology and a characteristic color after Eosin and Hematoxylin staining on the sections of human lungs: they have a disk-like shape and stained pink by H/E (Figures 2A and 2B).

Using this profile of adult RSC cells as “Identikit”, we searched again the literature on mouse lung development. We were lucky enough to find some pictures showing the presence of RSC-like cells at cellular level in the developing mouse lungs: in Figure 1E of an article published by Bellusci et al. [4], clusters of red cells could be clearly seen in the developing alveoli of a normal mouse lung at E17.5 (Figure 1E). Interestingly, the RSC-like cells disappeared in the lung of a transgenic mouse over-expressing Sonic hedgehog (Shh) driven by the surfactant protein-C (SP-C)-enhancer/promoter (Figure 1E), suggesting that the over-expression of Shh had an inhibitory effect on the development of RSC-like cells. Furthermore, at birth, the lungs of the transgenic mouse were paler and smaller than that of normal mouse (Figure 1D).

In a similar experiment, Dharmadhikari et al. [5] created transgenic mice over-expressing conditionally Foxf1, a downstream effector of Shh, in the endothelial and hematopoietic lineages. Conditional Foxf1 over-expression caused lethal lung hypoplasia and vascular defects in mice. The phenotype of the conditional Foxf1 over-expression was lung–specific, since other organs including the heart and liver, did not show any hematomas or evidence of blood pooling. Similar to the over-expression of Shh, the mouse lungs with Foxf1 over-expression was also much paler and smaller than that of the normal mice (Figure 1C). Once again, RSC-like cells could be seen in the alveolar spaces of the normal mouse lungs (Figures 2A and 2B), but disappeared in the lungs of transgenic mice.

Thus, these two independent studies by over-expressing two different genes of Shh signaling pathway showed an identical phenotype. Our interpretation of these results is that this color change and the hypoplasia of the lungs reflect a significant reduction of the number of RSC-like cells in the transgenic mice; Shh signaling pathway might negatively regulate the development of RSC-like cells in mouse lungs. In our opinion, it was the absence of RSC-like cells in transgenic mice, in both cases, led to the abnormal development of the mouse lungs and the death after birth due to respiratory failure. This conclusion is consistent with the observation of the authors: No obvious macroscopic differences were seen at E15.5, 16.5, 17.5 and 18.5 dpc between transgenic and normal lungs. Both the size and the wet weight after fixation and dehydration of the normal and transgenic lungs were similar. However, a clear difference was seen at birth. The two transgenic lungs obtained were smaller than normal (Figure 1D) and about half of the wet weight (an average of 39.3 ± 4 mg from four normal lungs compared with 18.6 mg for one of the SPC-Shh transgenic lungs) [4]. The turning point seemed to be E18.5, exactly the moment when the lungs turned red. Furthermore, we noticed a striking difference between the lungs of transgenic mice over-expressing Shh under SP-C promoter and that of mice over-expressing Foxf1 in the endothelial and hematopoietic lineages: at birth, the

former was still reddish; but the later was almost white! In other words, some RSC-cells might still be able to infiltrate the developing lungs at later time points, despite of a higher level of Shh in the lungs. In contrast, the RSC-like cells were completely absent in the lungs of transgenic mice over-expressing Foxf1 in the endothelial and hematopoietic lineages, suggesting a more severe phenotype. These results demonstrated, without ambiguity, that RSC-like cells made the mouse lung look red at E18.5. What is more, in the lung of transgenic mice over-expressing Foxf1, almost no Flk1-positive endothelial cell was present, suggesting an absence of capillary bed. These results further confirmed the crucial role of RSC-cells in Angiogenesis during mouse lung development.

Curiously, the authors of these two articles had, intentionally or inadvertently, overlooked this obvious change, namely the disappearance of the RSC-like cells in the alveolar lumen of transgenic mice. No single word on this phenotype! A possible explanation might be the briefness and transient nature of this phenomenon. On the other hand, it would be difficult, if not impossible, to make sense of this phenotype within the existing theoretical framework.

## Ontogeny of Lung Macrophages

Based on the previous results [6-9], Tan and Krasnow carried out a systematic study of lung macrophage development in mice [10]. They demonstrated that there are three distinct lineages of lung macrophages, originated independently from York Sac, Fetal liver and Bone marrow that arrive at different times, reside in different locations, renew in different ways and show little or no interconversion. Since we showed in our previous article, RSC cells have the ability to give rise to alveolar macrophages, RSC-like cells may be related to one of these three lineages described in the literature. According to Tan and Krasnow, the macrophage precursors (F4/80+) derived from the bone marrow arrive and expand in the mouse lungs after birth. Thus, we could rule out their possible involvement in color change of the mouse lungs at E18.5. The macrophage precursors from York Sac start to arrive and expand from E10, and they have a peripheral localization. Therefore, the timing and the location of this population of York Sac derived Macrophage progenitors do not match neither. The last possible candidate would be the fetal liver derived Mac2-positive progenitor cells.

We would argue that the fetal liver derived Mac2 positive progenitors may be the embryonic RSC cells in mouse.

- The timing seems to be right. According to Tan and Krasnow, the fetal liver derived Mac2 positive progenitor arrive and expand in the mouse lungs from E14 onwards. But we could further precise the time of arrival of RSC-like cells in the developing mouse lungs. If we look carefully at the histological data depicted in the article by Bellusci et al. [4], the RSC-like cells may have arrived in the mouse lung after E16.5, since no RSC-like cell was present in the alveolar lumen at E16.5 (Figure 1E). Consistent with this observation at the cellular level, the expression of genes involved in cell adhesion and migration (PC2pos) was picked at E16.5 and E17.5 of the developing mouse lungs (Figure 1D), presumably reflecting the arrival en masse of the RSC cells during this period.
- Shortly after the mouse lung becoming red at E18.5, there was a wave of expression of genes implicated in Angiogenesis/ Alveolarization between E18.5 and P2 (Figure 1F). In fact, Alveolarization and Angiogenesis are the two sides of the same coin, namely the formation of septa during the mouse lung development. Alveolarization and Angiogenesis are intrinsically linked and coordinated during septum formations [6]. That may be the reason why Alveolarization and Angiogenesis showed the same gene expression profiles. Moreover, these two waves of gene expressions seemed to be correlated with the primary and the secondary septations (Figure 1). In our previous article, we also suggested that RSC cells might play a role in Angiogenesis. Thus, all these data fit perfectly together: the arrival of RSC-like cells in the developing mouse lung from E17.5 onwards not only turned the mouse lungs red, but also initiated a phase of Angiogenesis and Alveolarization at saccular and alveolar stages, and the subsequent normal lung development.

Taken together, we believe that the fetal liver derived Mac2 positive progenitors and mouse RSC cells might be the same cells. This hypothesis would provide a simple and unified framework which could explain a variety of experimental results in a coherent way, ranging from anatomy, histology, cell biology, genetics, and molecular biology, developmental biology, etc.

As we suggested in our previous article, RSC cells are much more versatile than being merely the progenitor cells of alveolar macrophages. Thus, it would be indeed more appropriate to call this population of red cells, Red Soma Cells.

We are aware that our hypothesis may not be compatible with certain experimental data or with certain existing theories. For example, it has been proposed that the mature differentiated macrophages may have a self-renewal potential similar to that of stem cells, and they can maintain their population independent of hematopoietic stem cells.

Furthermore, this process may not require progenitors, since the mature macrophages can proliferate in response to specific stimuli indefinitely and without transformation or loss of functional differentiation [11].

But a recent article seems to contradict this notion: the human CD34+ hematopoietic stem and progenitor cells (HSPCs) could generate all three macrophage populations, occupying separate anatomical niches in the lung of a humanized mouse model [12].

## Discussion

It's undeniable that the human CD34+ hematopoietic stem and progenitor cells (HSPCs) have the ability to generate all three macrophage populations in a humanized mouse model.

Now the question is: Does such event really happen during the mouse or human development? As being pointed out by the authors, the MISTRG model does not recapitulate early stages of lung macrophage development in humans due to the absence of human fetal hematopoiesis and human macrophage precursors at birth [12].

Based on the results reported by Dharmadhikari et al. [5], it looks like the RSC-like cells constitute the unique cell type that could turn the developing mouse lungs red. The mouse embryos conditionally over-

expressing Foxf1 were red-colored, meaning that other hematopoietic stem cells, presumably those in the Bone marrow and in the Umbilical cord blood were present and functional. But, they couldn't rescue the phenotype of lethal lung hypoplasia and vascular defects in transgenic mice with conditional Foxf1-overexpression. Nevertheless, it remains to be determined if macrophages were present in the lungs of these transgenic mice.

## Conclusion

Taken together, we conclude that the Red Soma Cells, probably originated from fetal liver, constitute a unique cell population which might play critical roles in mouse lung development.

## Acknowledgement

We thank all the members of Epithelix' team for their supports. In particular, Samuel Constant and Ludovic Wiszniewski for financial support and scientific discussions.

## Conflict of Interest

The authors declare no conflict of interest.

## References

1. Huang S, Lopez C (2021) What makes our lungs look red? bioRxiv.
2. Galambos C, Ng YS, Ali A, Noguchi A, Lovejoy S, et al. (2002) Defective pulmonary development in the absence of heparin-binding vascular endothelial growth factor isoforms. Am J Respir Cell Mol Biol 27:194-203.
3. Beauchemin KJ, Wells JM, Kho AT, Philip VM, Kamir D, et al. (2016) Temporal dynamics of the developing lung transcriptome in three common inbred strains of laboratory mice reveals multiple stages of postnatal alveolar development. Peer J 4:2318.
4. Bellusci S, Furuta Y, Rush MG, Henderson R, Winnier G, et al. (1997) Involvement of Sonic hedgehog (Shh) in mouse embryonic lung growth and morphogenesis. Dev Camb Engl 124:53–63.
5. Dharmadhikari AV, Sun JJ, Gogolewski K, Carofino BL, Ustiyan V, et al. (2016) Lethal lung hypoplasia and vascular defects in mice with conditional Foxf1 overexpression. Biol Open 5:1595-1606.
6. Loscertales M, Nicolaou F, Jeanne M, Longoni M, Gould DB, et al. (2016) Type IV collagen drives alveolar epithelial–endothelial association and the morphogenetic movements of septation. BMC Boil 14(1):1-21.
7. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, et al. (2010) Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 330:841-845.
8. Hoeffel G, Wang Y, Greter M, See P, Teo P, et al. (2012) Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac–derived macrophages. J Exp Med 209:1167-1181.
9. Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, et al. (2013) Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. J Exp Med 210:1977-1992.
10. Tan SY, Krasnow MA (2016) Developmental origin of lung macrophage diversity. Dev Camb Engl 143:1318–1327.
11. Sieweke MH, Allen JE (2013) Beyond stem cells: self-renewal of differentiated macrophages. Science 342.
12. Evren E, Ringqvist E, Tripathi KP, Sleiers N, Rives IC, et al. (2021) Distinct developmental pathways from blood monocytes generate human lung macrophage diversity. Immunity 54:259-275.
13. Aziz A, Soucie E, Sarrazin S, Sieweke MH (2009) MafB/c-Maf deficiency enables self-renewal of differentiated functional macrophages. Science 326:867-871.