inside blood commentary

16 NOVEMBER 2017 | VOLUME 130, NUMBER 20

HEMATOPOIESIS AND STEM CELLS

Comment on Zhang et al, page 2161

Miss Piggy on the catwalk again

Anna Rita Migliaccio ICAHN SCHOOL OF MEDICINE; UNIVERSITÀ DI BOLOGNA

In this issue of *Blood*, by generating a novel dual oxidase 2 (*DUOX2*) mutation responsible for congenital hypothyroidism (CH) in pigs, Zhang et al identify Krüppel-like factor 9 (KLF9) as the mediator for the regulation exerted by the thyroid gland on hematopoiesis.[1]

The domestic pig has been the "best friend" of medical studies for centuries. In fact, although Greeks cherished dissection of human cadavers to study anatomy for medical purposes, Romans considered the body of the deceased untouchable and forbade human dissections. Claudius Galenus of Pergamum (AD 129-216), who was the physician to several Roman emperors, prized the use of animals, most of all simians, as surrogate models to study the human body and wrote many textbooks on this subject. These studies soon identified profound similarities between the anatomy of pigs and that of humans, leading to pig dissection as the model of choice to teach human anatomy. Of the numerous textbooks that must have been written on this subject at that time, *Anatomia Porci*, written in the middle of the eleventh century by the Jewish physician Cophonis of the Schola Medica Salernitana (a medical school founded in the ninth century in southern Italy[2]) has survived and is still currently referenced.

Pigs, however, lack the cecal appendix, and it was soon recognized that they are poor models on which to practice its surgical removal in the case of appendicitis. In addition, in the 1400s, during the Renaissance movement, the importance of the dissection of human cadavers for artistic and medical purposes was rediscovered. These studies were pioneered by Mondino de Luzzi (1270-1326), a physician and anatomist from the University of Bologna whose *Anathomia Mundini* (manuscript 1316; first printed in 1478) was the first European book written since classical antiquity entirely devoted to human anatomy.[3]

The use of porcine experimental models to study hematopoiesis goes back to the 1950s and 1960s when these animals, because of their size being similar to that of humans, were used to study radiation-induced leukemia. Studies in pigs also provided the first evidence for the presence of erythroid-stimulating activity in liver and kidney (which were probably represented by interleukin-6 and erythropoietin, respectively) and for the changes in iron homeostasis occurring in response to anemia.[4-6] However, studies in pigs are costly and technically challenging, and, in spite of the development of miniaturized easy-to-handle strains, studies on hematopoiesis in pigs are rare.

More recently, the sequencing of the 2.8 billion base pairs of the entire swine genome published by the Swine Genome Sequencing Consortium has provided a genetic basis for the striking similarities between the anatomies of pigs and humans. The porcine genome contains 21 640 coding genes and exhibits important similarities with the human genome.[7] Most notably, comparison of predicted porcine protein sequences with their human orthologs identified 112 positions in which the porcine protein has the same amino acid that is implicated in a human disease.[7] This recognition has revitalized the importance of the pig as a biomedical model. In fact, based on the similarities between the anatomy, physiology, and genetics of pigs and humans, and thanks to the development of more efficacious immunosuppressive therapies, investigators are developing innovative xenotransplantation protocols using porcine organs (mainly heart and liver) to treat organ failure in humans. In addition, spontaneous mutations already existing, or transgenic and knockout models obtained by molecular engineering technology (including somatic nuclear-cloning procedures) have generated porcine models in which to study several human diseases (obesity, diabetes, and even dyslexia, Parkinson disease, and Alzheimer disease).[8]

The study by Zhang et al in this issue used ethylnitrosourea-induced mutagenesis, followed by whole-genome sequencing, to generate a hypomorphic recessive mutation in the gene encoding DUOX2 in Bama miniature pigs.[1] This pig model expresses a phenotype similar to that of the genetic endocrine disease CH, which includes a mild anemia and impaired immune functions. Although the single amino acid mutation D409D in *DUOX2* induced in pigs is different from the single mutations in *DUOX2* present in human cases of CH, the D409D-DUOX2 protein expresses reduced levels of H_2O_2 synthetic activity and impairs biosynthesis of thyroid hormone (TH) in a fashion similar to that of the mutant proteins found in the human disease. Zhang et al used this model to identify that the transcription factor KLF9 is a major target of TH in numerous cellular systems. In addition, by using a knockout approach, they demonstrate that KLF9 regulates erythroid cell production in zebrafish. This is the first article that provides evidence for an active role of KLF9 in erythropoiesis.

The study raises many questions, the most important of which is whether KLF9 affects red cell production directly or indirectly

by acting on the niche. Although belonging to the 17-member KLF family of proteins, which contains highly similar zinc-finger regions, KLF9 segregates to a specific subgroup and its functions may not be expected to be functionally redundant with that of other members of the family, such as KLF1.[9] So, it is unlikely that the functions of KLF9 in erythropoiesis overlap with those of KLF1. Recent studies have demonstrated that terminal erythroid maturation of human erythrocytes in culture (and, in particular, the final enucleation step) requires, in addition to erythropoietin, the presence of TH.[10] The fact that KLF9 is downstream to the TH axis[1] suggests that KLF9 represents the long-sought factor that regulates enucleation downstream to GATA1 and KLF1 during terminal erythroid maturation. Further studies are now necessary to detail the biology of the TH/KLF9 axis in erythropoiesis.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

REFERENCES

1. Zhang Y, Xue Y, Cao C, et al. Thyroid hormone regulates hematopoiesis via the TR-KLF9 axis. *Blood*. 2017;130(20):2161-2170.

2. Corner GW. Anatomical Texts of the Earlier Middle Ages. Washington, DC: Carnegie Institution of Washington; 1927.

3. Olmi G. Representing the Body: Art and Anatomy from Leonardo to Enlightenment. Bologna, Italy: Bononia University Press; 2006.

4. Heinle RW, Welch AD, Pritchard JA. Essentiality of both the antipernicious anemia factor of liver and pteroylglutamic acid for hematopoiesis in swine. *J Lab Clin Med*. 1948;33(12):1647.

5. Peschle C, Tallarida G, Semprini A, Ravetta M, Condorelli M. Research on the renal erythropoietic factor. I. Behavior of erythropoietic activity in rats treated with purified extracts of swine kidney with renin activity [in Italian]. *Boll Soc Ital Biol Sper*. 1967;43(13):755-759.

6. Bush JA, Jensen WN, Ashenbrucker H, Cartwright GE, Wintrobe MM. The kinetics of iron metabolism in swine with various experimentally induced anemias. *J Exp Med*. 1956;103(1):161-171.

7. Groenen MA, Archibald AL, Uenishi H, et al. Analyses of pig genomes provide insight into porcine demography and evolution. *Nature*. 2012;491(7424):393-398.

8. Prather RS, Lorson M, Ross JW, Whyte JJ, Walters E. Genetically engineered pig models for human diseases. *Annu Rev Anim Biosci*. 2013;1(1):203-219.

9. Bieker JJ. Krüppel-like factors: three fingers in many pies. *J Biol Chem*. 2001;276(37):34355-34358.

10. van den Akker E, Satchwell TJ, Pellegrin S, Daniels G, Toye AM. The majority of the in vitro erythroid expansion potential resides in CD34(-) cells, outweighing the contribution of CD34(+) cells and significantly increasing the erythroblast yield from peripheral blood samples. *Haematologica*. 2010;95(9):1594-1598.

DOI 10.1182/blood-2017-10-809640

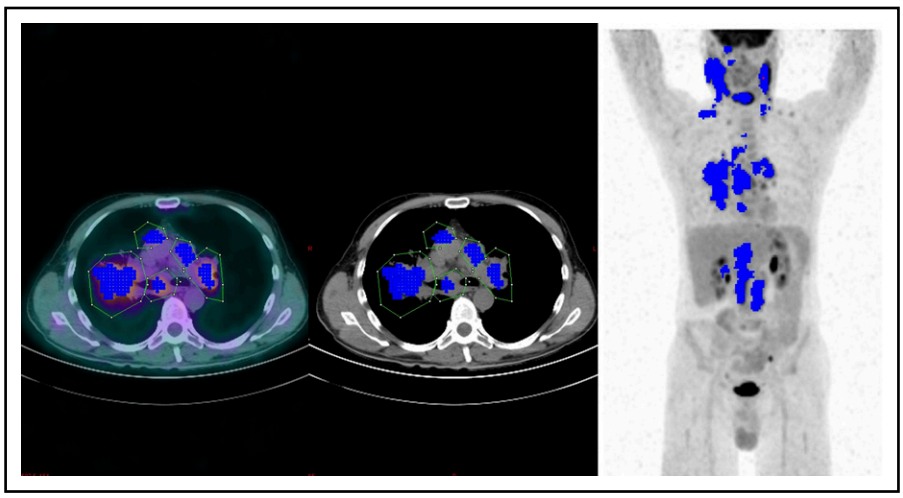
Comment on Moskowitz et al, page 2196

Relapsed/refractory HL: FDG-PET is the trump card

Andrea Gallamini ANTOINE-LACASSAGNE CANCER CENTER

A number of disease- or host-related markers have been proposed to predict treatment outcome in relapsed/refractory Hodgkin lymphoma (HL). In this issue of *Blood*, Moskowitz et al demonstrate that fluorodeoxyglucose (FDG)-positron emission tomography (PET)/computed tomography (CT) performed in sequence during second-line treatment recapitulates all of them.[1]

In the era of new drugs, high-dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) remains the best treatment option for relapsed/refractory HL. Intensity and duration of second-line salvage therapy do not affect treatment outcome if HDC plus ASCT follows chemotherapy.[2] Quite recently, an international consortium for prognostic factors modeling in relapsed/refractory HL (the RisPACT consortium) retrospectively evaluated the impact of 23 risk factors (RF) on ASCT outcome in 546 patients with relapsed/refractory HL in 9 prospective trials.[3] Stage IV, time to relapse ≤3 months, Eastern Cooperative Oncology Group performance status ≥1, a bulk nodal lesion >5 cm, and an inadequate therapy response, assessed with a CT or PET/CT before ASCT, proved to be, in multivariate analysis, the only RF predictive of progression-free survival. Different groups have demonstrated that interim PET (iPET) before ASCT in relapsed/refractory HL had a predictive value (PV) independent from other RFs,[4] similar to that during doxorubicin, bleomycin, vinblastine, dacarbazine treatment, where iPET proved the prognostic marker with the highest PV, irrespective of other RFs included in the International Prognostic Score.[5] The physiopathological mechanism underpinning this very high PV in HL is complex and incompletely understood. The great avidity for FDG reported in HL has been allegedly attributed to the high glycolytic activity of the microenvironment (ME) cells. In the latter, as in neoplastic cells, the glycolysis proved as high as 200 times that of normal tissue. ME is, by far, the largest cellular fraction in HL tissue, and its metabolic activity is sustained by a cytokine network produced by few neoplastic Hodgkin and Reed-Sternberg (HRS) cells. On the



Tumor segmentation in an HL patient showing 5 sizeable mediastinal lymph nodes (courtesy of Salim Kanoun).