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Age-related reduction of structural complexity in spleen hematopoietic tissue architecture in mice

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ABSTRACT

The effects of aging on structural complexity in hematopoietic tissue are unknown. In this work, in a mouse experimental model, we report the age-related reduction of spleen hematopoietic tissue (SHT) complexity. Spleen tissue was obtained from the total of 64 male Swiss albino mice divided into 8 age groups: newborns (0 days old), 10 days, 20 days, 30 days, 120 days, 210 days, 300 and 390 days old. SHT was stained using conventional hematoxylin/eosin, and DNA-binding toluidine blue dyes. Fractal dimension as an indicator of cellular complexity, and lacunarity as indicator of tissue heterogeneity were determined based on the binarized SHT micrographs. Results indicate that fractal dimension of mice spleen hematopoietic tissue decreases with age, while lacunarity increases. These changes/trends have been detected in SHT stained both with toluidine blue and conventional hematoxylin/eosin. Fractal dimension was negatively correlated with lacunarity. The detected reduction in complexity suggests that age-related structural changes are present in mouse SHT both in general tissue architecture and progenitor cell DNA.

1. Introduction

Hematopoiesis is a process in which immature precursor cells (hematopoietic stem cells) differentiate into specialized blood cells such as erythrocytes and leukocytes. It is known that hematopoietic tissue (HT) undergoes age-related changes regarding its functional status. Accumulation of DNA damage and telomere dysfunction are important causes of hematopoietic stem cell loss (Wang et al., 2011). It is assumed that with age, the stem cell functions gradually decrease, which is followed by the increase of the percentage of fat tissue within the bone marrow (one of the locations where hematopoiesis may take place) as well as lower blood interleukin levels (Tuljapurkar et al., 2011). Apart from intrinsic factors, microenvironment also plays an important role in hematopoietic stem cell (HSC) aging, since these cells cannot express their full potential without an adequate communication with surrounding non-stem cells. Some of these microenvironment factors include, but are not limited to reduced adherence of HSCs with stromal cells, as well as the accumulation of adipocytes and other cells that can influence the HSC function (Woolthuis et al., 2011; Xing et al., 2006). Whatever the reason for the impaired HSC function, it is clear that changes in hematopoietic tissue structural organization may have a major impact on the ability of aged HSCs to differentiate into specialized blood cells.

In most organisms, the primary location where hematopoiesis takes place is the bone marrow. During embryological development, as well as in some pathological conditions, the spleen, among other organs and tissues, may also act as a hematopoietic site. In some animal species, such as mice, this extramedullary hematopoiesis is a physiological property of both embryonic and adult spleen tissue. Mouse spleen hematopoietic tissue is especially active in erythropoiesis. Compared to the bone marrow hematopoietic tissue (BMHT), rodent SHT can in some circumstances reach up to 50% of its erythropoiesis output. In conventional histological sections, numerous erythroid progenitor cells, assembled in aggregates, can be seen in of mouse subcapsular spleen tissue (Fox, 2007; Pantic et al., 2013; Suttie, 2006).

The mouse spleen HSCs are somewhat morphologically and functionally similar to bone marrow HSCs (Morita et al., 2011). However, unlike in the bone marrow, the effects of aging on structural and cytoarchitectural characteristics of this spleen hematopoietic tissue remain largely unknown. Also, in mouse spleen HT it is unknown to

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what extent microenvironment and inter-cellular communication influences potential age-related functional decrease.

There are several ways to assess structural organization and cell–cell communication within a tissue. One of the methods today commonly used for evaluation of tissue structural and ultrastructural features is fractal analysis (FA). Fractal dimension and lacunarity of binarized tissue micrographs as FA parameters are important indicators of tissue complexity and heterogeneity (Gilmore et al., 2009; Gould et al., 2011; Kimler et al., 2004; Tambasco et al., 2009). FA has so far been successfully applied in complexity analysis of various tissues and organs, including the liver (Dioguardi et al., 2006; Olefirenko et al., 2009), brain tissue (Milosevic et al., 2010; Ristanovic et al., 2010) and blood vessels (Lorthois and Cassot, 2010). In this study, based on a sample of 64 Swiss albino mice divided into 8 age groups, we present the results that complexity of mouse SHT, measured by the values of fractal dimension, decreases with age, while lacunarity as a measure of tissue heterogeneity increases. These changes/trends have been detected both in hematoxylin/eosin and DNA-binding toluidine blue stained tissue and indicate that aging substantially affects mouse spleen HT integrity, which may be followed by adequate functional changes.

To our knowledge, this is the first study to investigate potential age-related changes in spleen hematopoietic complexity and heterogeneity. It is also the first to apply fractal dimension in evaluation of SHT structural organization.

2. Material and methods

Spleen tissue was obtained from 64 healthy male Swiss albino mice, divided into 8 age groups: newborns (0 days old), 10 days, 20 days, 30 days, 120 days, 210 days, 300 and 390 days old. The breeding and time follow-up (13 months for the oldest animal) were carried out in the same environmental conditions (standard laboratory diet, ambient temperature of 22 °C, natural light cycle). No veterinary illnesses of any sort were detected during the follow-up period.

The study conformed to the guiding principles for the care and use of vertebrate animals in research and training, established by the American Physiological Society (APS) in 1953, and revised by APS on July 16, 2010. The study protocol received authorization by the Ethical Commission for laboratory animal welfare of the University of Belgrade, Faculty of Medicine, and the Ministry of Agriculture, Trade, Forestry and Water Management, Republic of Serbia (Decision No. 323-07-03985/2012-05/1).

The spleen tissue was fixated Carnoy solution and embedded in Paraplast® was cut into five-micrometer thick section, mounted on glass slides, and stained using hematoxylin/eosin (H/E) and DNA-binding toluidine blue techniques. Digital micrographs of hematopoietic tissue were made using Olympus C-5060 Wide Zoom digital camera instrument, with an oil immersion objective (×1000 magnification) of Olympus BX41 light microscope. In each histological section, the visualized and imaged portion of the spleen hematopoietic tissue was located approximately 100–300 μm beneath the spleen capsule. Figs. 1a and 2a represent the examples of visualized SHT segments in a newborn animal (H/E stain) and 390-day-old animal (toluidine blue stain) respectively. For each animal, micrographs were acquired in RGB layout and converted to binary format (Figs. 1 and 2). The experimental design regarding tissue fixation, embedment and micrograph acquisition was similar to the studies previously published by our laboratory (Pantic and Pantic, 2012; Pantic et al., 2012a,c). The two major parameters of fractal complexity analysis, fractal dimension and lacunarity were calculated for each micrograph.

2.1. Toluidine blue staining protocol

Toluidine blue staining was performed as previously described (Lilli, 1965). Briefly, tissue sections, after being mounted on glass slides, were washed with xylol (2 × 15 min), 100% ethanol (2 × 2 min), 96% ethanol (2 min), and 70% ethanol (5 min). Afterwards the sections were dyed with 0.5% toluidine blue in 0.01 N HCl solution for 30 min. Finally, the sections were again washed with 100% ethanol and xylol (15 min). Toluidine blue is a basic thiazine metachromatic dye which interacts with DNA within the cell nucleus (Ilanchelian and Ramaraj, 2011; Lilli, 1965; Passmore and Killeen, 1996).

2.2. Preliminary analysis of the spleen follicular tissue complexity

In addition to the subcapsular spleen hematopoietic tissue analysis we also performed preliminary calculation of follicular complexity within the white pulp adjacent to the analyzed segments of SHT. Briefly, for each animal, a primary follicle in the immediate proximity of subcapsular SHT was visualized and a digital micrograph was created similarly as previously described. As for the SHT, the values of fractal dimension and lacunarity were determined for each primary follicle.

3. Theory/calculation

3.1. Fractal dimension calculation

Fractal dimension of binarized micrographs was determined using box-counting method, as previously described (Lopes and Betrouni, 2009). This technique, like many other fractal analysis methods, is based on so called Richardson–Mandelbrot Plot (Falconer, 2003) where different measures of a set are plotted against the box size (r) on a double logarithmic axis and fractal dimension is calculated from the slope of the regression line (Falconer, 2003).

Box-counting method calculates the box fractal dimension (D_B) of the micrograph, which is also known as capacity dimension or Kolmogorov dimension (Fernández and Jelinek, 2001). In brief, the digitized image is covered with a grid of square cells. Box fractal

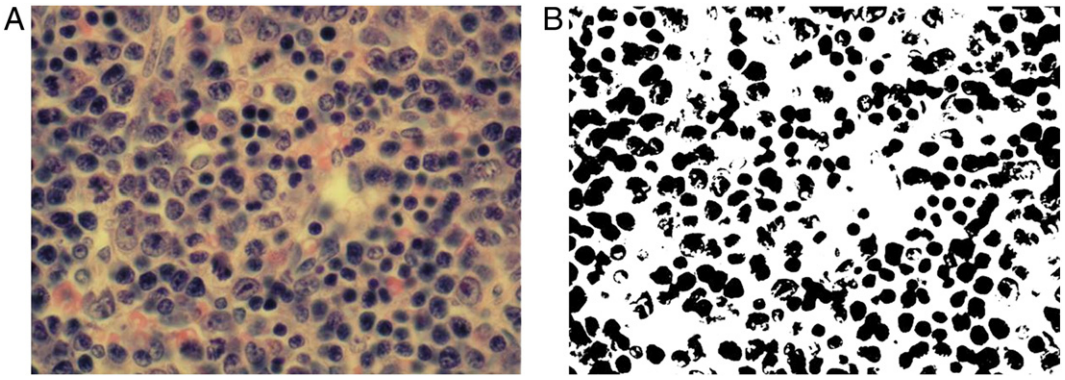

Fig. 1. Digital micrograph of hematoxylin/eosin stained spleen hematopoietic tissue (A, newborn animal). Before fractal analysis, micrographs were converted to binary format (B).