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The Neutrophil Nucleus and Its Role in Neutrophilic Function

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ABSTRACT

The cell nucleus plays a key role in differentiation processes in eukaryotic cells. It is not the nucleus in particular, but the organization of the genes and their remodeling that provides the data for the adjustments to be made according to the medium. The neutrophil nucleus has a different morphology. It is a multi-lobed nucleus where some researchers argue no longer function. However, studies indicate that it is very probable the occurrence of chromatin remodeling during activation steps. It may be that the human neutrophil nucleus also contributes to the mobility of neutrophils through thin tissue spaces. Questions like these will be discussed in this small review. The topics include morphology of human neutrophil nucleus, maturation process and modifications of the neutrophil nucleus, neutrophil activation and chromatin modifications, causes and consequences of multi-lobulated segmented morphology, and importance of the nucleus in the formation of neutrophil extracellular traps (NETs). J. Cell. Biochem. 116: 1831–1836, 2015.

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The view that the cell nucleus is only a way of protecting the genome and nuclear bodies is outdated. The nucleus regulates the gene expression at the levels of chromatin, transcription, RNA processing, and RNA export [Wieslander, 2004]. Further, in some cases, nuclear proteins participate on cytoplasm metabolic pathways. One example is the formation, in the nucleus, of CMP-Sialic Acid, important in the process of sialic acid generation for a large number of cell surface proteins like PSGL-1, for example, which initiates intracellular events that stimulate neutrophil firm adhesion to the endothelium [De Fátima et al., 2005; Zarbock and Ley, 2008].

In addition, the cell nucleus can assume a diverse array of morphologies that usually correlate to functions and mechanisms. The human neutrophil nucleus is a notable example of this nuclear shape variation presenting a segmented multi-lobulated morphology. This cell is the most abundant leukocyte capable of performing a wide array of functions, such as phagocytosis, secretion of cytokines, production and self-protection against ROS, cell adhesion, cytoskeleton reorganization, formation of extracellular traps that can kill pathogens, among many others [Cassatella, 2003; Brinkmann et al., 2004; Teles, 2007].

The cell nucleus particular morphologies probably enhance many cell functions. But how does the nucleus of a cell such as human neutrophils, which is considered as terminally differentiated cells by one portion of the scientific community, may contribute to the cell functionality? The multi-lobulated morphology of the human neutrophil nucleus could be involved in the enhancement of the cell motility through the tight tissue spaces [Hoffmann et al., 2007] and in the enhancement of the phagocytosis since the segmented nucleus theoretically offers a larger space to accommodate the pathogens in the cytoplasm.

In addition, many authors consider that the neutrophil nucleus has not "lost" functionality in human neutrophils [Malcolm et al., 2003; Tsukahara et al., 2003; Zhang et al., 2004]. The processes involved in neutrophil activation and functionality are very complex wherein the quiescent neutrophils are very different from activated neutrophils. So, how a cell could develop this high level of differentiation without the contribution of the cell nucleus? Zhang et al. [2004] found that there are a broad and vigorous set of alterations in gene expression of activated human neutrophils compared with quiescent stage.

One of those mysteries that still prevail and perhaps one of the most intriguing of the biology is the cell differentiation process [Rastogi, 2003]. As well as the mapping of the human genome was

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the first step to study the transcription products, understanding the responses of the nucleus to certain stimuli can be considered the first step to elucidate the differentiation process of several cell types. The importance of the nucleus in the process of differentiation is very clear. Enucleated cells like human erythrocytes, for example, not are able to differentiate. Also, many bacteria had a "limited differentiation" by conjugation. Many protozoans parasites, however, had a tremendous power to differentiate, as well as human neutrophils [Lopes, 2000; Tsukahara et al., 2003; Zhang et al., 2004].

Our objective is to describe some basic aspects of the neutrophil nucleus: correlating the nuclear shape to the organelle functions and the underlying mechanisms. These three aspects, morphology, physiology, and biochemistry will be covered in the discussion of the neutrophil maturation, the formation of the segmented nucleus, the cellular activation, and the cell death mechanisms.

DEVELOPMENT

MORPHOLOGY OF HUMAN NEUTROPHIL NUCLEUS

The neutrophil nucleus occupies nearly a quarter of the cytoplasmic volume [Hodges et al., 2003]. The typical nuclear structure includes nuclear lobes, connecting segments and, in some cases, nuclear appendages [Sanchez and Wangh, 1999]. The reason for the occurrence of these appendages in only a percentage of the neutrophils is still unknown.

In human neutrophils the nuclear lobes ranges from 2 to 6. Usually, healthy donors show neutrophils with 3–4 lobes, but this number can change during neutrophil activation [Campbell et al., 1995; Olins and Olins, 2005b; Fuchs et al., 2007; Hoffmann et al., 2007]. Each lobe has approximately 2 μm of diameter and presents flexibility in its structure, enhanced by thin connections and by molecular structure of the neutrophil nuclear membranes [Campbell et al., 1995; Brinkmann and Zychlinsky, 2007; Rowat et al., 2013].

These connecting segments are found between two lobes; most of them range in width from 0.2 to 0.5 μm [Sanchez et al., 1997]. So, they range from a very thin length to a more gradual constriction of the nucleus. In addition, they are able to stretch and attenuate extensively as the cell moves and can also bend or slack to accommodate the movements of adjacent lobes [Campbell et al., 1995].

Finally, this inter-lobe filaments present barriers to diffusion of fluo3–Ca2+ and lucifer yellow, for this reason Hodges et al. suggests that they are heavily packed with chromosomal material and, before that, Campbell et al. found that they contain DNA [Campbell et al., 1995; Hodges et al., 2003].

As we stated, the neutrophil nucleus is not only composed by lobes and connecting filaments. There is a peculiarity related to the sex chromosomes. Healthy males have approximately 25% of the neutrophil nuclei with the Y chromosome isolated in a compartment known as appendage [Karni et al., 2001]. In females, the frequency decreases (17%) and the appendage compartment has a morphology that is slightly different (drumstick morphology). Maybe the reason is associated with the differences between X and Y isolated chromosomes [Davidson and Smith, 1954; Sanchez et al., 1997]. Interestingly, Sanchez and Wangh found that the frequency of drumstick appendage formation increases with the extent of nuclear segmentation, suggesting that the same mechanisms that mediate appendage formation may also control filament assembly during maturation process [Sanchez and Wangh, 1999].

About nucleolus, some works defend the lack of this nuclear subcompartment (presence and activity) in mature neutrophils. Salati et al. failed to detect the presence of nucleoli in human neutrophils using silver staining techniques of nucleolus regions. In addition, they affirm that neutrophil lack the capacity to repair double- and single-strand DNA breaks [Salati et al., 2007].

On the other hand, as we inferred, some authors defend many alterations in gene expression in mature human neutrophils [Malcolm et al., 2003; Tsukahara et al., 2003; Zhang et al., 2004]. In addition, Smetana et al. found nucleoli presence in the final stages of maturation of human granulocytes (metamyelocytes, band segmented form, granulocyte) with a reduced size (~40% of diameter presented by myeloblasts) [Smetana et al., 2011]. The mere permanence of nucleoli in neutrophils, even in small sizes, suggests that they are still active.

MATURATION PROCESS AND MODIFICATIONS OF THE NEUTROPHIL NUCLEUS

Human neutrophils are generated by hematopoietic cells from a lineage known as myeloblasts which have a large oval nucleus with loose chromatin occupying almost all the cytoplasm. Myeloblasts differentiate into promyelocytes which can be recognized by their large diameter (~15 μm) and rounded nucleus. And then promyelocyte originates the myelocyte which have indented nucleus with condensed nuclear chromatin [Bainton et al., 1971; Teles, 2007].

After those stages, the metamyelocytes, bands, and mature PMNs are non-dividing cells included into post-mitotic phase [Bainton et al., 1971]. While the maturation of metamyelocytes proceeds, the nucleus becomes increasingly invaginated or horseshoe shaped, lacks nucleoli, and contains moderately condensed chromatin masses beneath the nuclear envelope [Kuijpers and Roos, 2001]. At the stage of bands, the nuclear indentation continues and this cell differs from metamyelocytes when more than a half of the diameter of the nucleus presents indentations. When at least one area of nuclear constriction becomes a thin filament, the cell reached the final stage of the process of maturation and will be called neutrophil or segmented PMN [Sanchez et al., 1997; Teles, 2007].

Summarizing, at the course of human neutrophil differentiation, the round nucleus of the promyelocyte precursor undergoes profound structural changes including nuclear segmentation and accumulation of peripheral heterochromatin, especially during the post-mitotic phase [Campbell et al., 1995; Zwerger et al., 2008].

NEUTROPHIL ACTIVATION AND CHROMATIN MODIFICATIONS

Activation is the process of differentiation of mature circulating quiescent neutrophils to activated neutrophils that phagocyte pathogens and release cytotoxins in many organ tissues [Teles, 2007; Botha, et al., 1995].

We do not find in the literature one work that proves the occurrence of chromatin remodeling during activation of a neutrophil. However, Zhang et al. [2004] practically does this by finding a broad and vigorous set of alterations in gene expression of

activated human neutrophils compared with quiescent stage as we inferred. Different genes being expressed in each state strongly suggest a chromatin remodeling.

In addition, Fuchs et al. [2007] found that upon stimulation the neutrophil euchromatin and heterochromatin homogenize. This finding also suggests that chromatin modifications happen during neutrophil activation. These findings attack the theory that the neutrophils are terminally differentiated cells.

During neutrophil extracellular trap formation we will discuss a dramatic alteration of the neutrophil chromatin, which will be used in these traps that can kill bacteria [Brinkmann and Zychlinsky, 2012].

CAUSES AND CONSEQUENCES OF MULTI-LOBULATED SEGMENTED MORPHOLOGY

Cell and organelle shape remain with many open questions in biological research. It is very likely that unusual nuclear morphologies are related with the features and functions that these cells perform [Hoffmann et al., 2007]. It is also a consensus that the origin of such shapes is determined by multiple factors, such as the arrangement of the many biomolecules contained in the respective compartments [Olins et al., 2008a,b]. Neutrophil multi-lobulated nucleus is quite different from most human cells, but what are the advantages of that? How is it assembled?

In the case of the neutrophils, it is important to consider that the nuclear morphology varies considerably between species. For example, most non-vertebrates and a few vertebrates like some reptiles (snakes and turtles) have mature neutrophils with round nuclei. However, some mammals like camels, hyenas, guinea pigs, and rabbits have a hypersegmented nuclei compared to human neutrophils. Elephants, many fishes, and birds like chicken have hyposegmented neutrophil nuclei, again compared to human neutrophils. Other species like mice and rats have neutrophils with ring-shaped nuclei. Finally, most of mammals and the frog Bufo vulgaris have neutrophils with lobulation comparable to humans [Hoffmann et al., 2007]. It is important to realize that different animal classes/species have different tissue densities and this factor may be involved in these dramatic nuclear morphology variations among species.

But why neutrophil nucleus acquires multilobulated morphology? Well, the human PMNs are the first cell type to arrive at inflamed sites after stimulus and can move more rapidly than any other cell type of organism [Brewer, 1972; Cassatella, 2003]. Ada L. Olins et al. published that the human granulocyte nucleus has a paucity of LINC complex proteins and lamins in the nuclear envelope (NE). In consequence, segmented neutrophil nucleus appears to be very malleable, as we inferred earlier [Olins et al., 2008a,b].

In addition, the serial-section electron microscopy of human neutrophils emigrating from venules demonstrates an elongation of the nucleus during trans-endothelial migration [Hoffmann et al., 2007]. Other studies further demonstrated that in vitro differentiated granulocytes (hypolobulated nuclei) exhibited deficient migration through membranes with 3.0 micrometers pores in response to chemoattractants [Gaines et al., 2008]. Moreover, it is easy to identify atypical and marked morphological changes in malignant tumor cell nuclei for example. It is known that these cells also have a high invasive power and can reach across several different human tissues [Campbell et al., 1995].

Altogether, these factors serve as examples which induce us to believe that the multi-lobulated neutrophil nuclei favors diapedesis and cell mobility during migration through tight tissue spaces [Hoffmann et al., 2007]. So, animals presenting segmented nuclei (and consequently easier tissue migration) might have faced an evolutionary advantage. However, Rowat et al. [2013] suggest that lamin A is very important on the ability of neutrophils to pass through micron-scale constrictions, and they suggest that the segmented nuclear morphology is less essential in this process.

There are still other consequences observed in the literature which probably occur due to nuclear segmentation. Nakayama and Yamaguchi [2005] suggest, by cell sorting analysis, that the cell cycle is delayed in S phase through multi-lobulation and this alteration could be associated to the disable of the mitosis process in granulocytes. In addition, we do not find works discussing the possibility of enhancement of phagocytosis promoted by multi-lobulated morphology but we imagine that it is a possibility.

About how the segmented morphology is acquired, it has been suggested a possible role of cytoskeleton since cytoskeletal proteins have been shown to undergo alterations in expression and/or distribution during the differentiation of granulocytic leukemia cells [Campbell et al., 1995]. However, treatment of isolated neutrophils with nocodazole and microfilament-poison cytochalasin-B has no effect on nuclear morphology. This result suggests that the effect of cytoskeletal inhibitors occurs during maturation rather than at the level of terminal differentiation [Sanchez et al., 1997]. Moreover, Sarria et al. [1994] suggest a role for the intermediate filament protein vimentin. Vim − SW-13 cells appeared to be frequently characterized by large folds or invaginations forming prominent lobes and clefts, whereas Vim + cells had a more regular or smooth nuclear shape. So in this case it appears that multi-lobulated morphology is linked to less cytoskeleton proteins configuration.

Accordingly, Olins and Olins [2004] observed a decrease in vimentin concentration during the differentiation of HL-60/S4 cells. In our laboratory Elaine et al. demonstrated, by proteomics procedures, an increase in the expression of vimentin in human neutrophils stimulated in vitro by PAF and some studies report that during activation the nucleus may suffer a hipolobulization [Hoffmann et al., 2007; Aquino, 2008]. These results support the idea that the vimentin has an influence in the induction of nuclear morphology segmentation in neutrophils and apparently, a lower concentration of vimentin favors multi-lobulation, but the role of vimentin in granulocyte structure and function is not clearly established as yet [Olins and Olins, 2005a].

Yet about cytoskeleton role, Olins and Olins found a close proximity of the centrosomal region (with centrioles) to the major invaginations of granulocytic HL-60 cells and within the central ring hole of granulocytic MPRO cells (mouse). They suggest that the nuclear envelope undergoes invaginations in the vicinity of the centrosome through cytoskeletal proteins that comprise a motor system (dynein) which binds to nuclear envelope and pulling towards the juxtanuclear centrosomal region, promoting the tensor force required to start the nuclear invagination [Olins and Olins, 2005a; Zwerger et al., 2008].

However, as we inferred earlier, they found a paucity of lamin A/C, B1, B2, LAP2b, and emerin (spectrin-like proteins) in nuclear envelope of granulocytes and it suggests that this bridging system may not be functional in neutrophils [Olins and Olins, 2005a; Olins et al., 2008b]. Small amounts may not mean there was no link, but, as yet, there is no evidence for cytoplasm dynein playing a role in granulocytic nuclear differentiation [Olins et al., 2008b] and Campbell et al. [1995] saw no bundles of actin or microtubules, or any other distinctive features in the cytoplasmic regions surrounding the connecting segments. Also, is probable that the nuclear composition changes widely during maturation. Maybe the concentration of spectrin-like proteins in nuclear envelope is favorable to establish the link with dynein motor system in some step of myeloid development.

As we inferred earlier, it has also been suggested a possible role performed by the NE, through lamins for example, the major architectural proteins of the nucleus proposed to play crucial role in determining nuclear shape and in maintenance of nuclear integrity [Galiová et al., 2008; Parnaik, 2008]. Neutrophil nucleus have lamin B but lack lamin A/C and rounded nucleus usually have all these lamin types. So the remodeling of the nuclear lamins must be involved in the nuclear segmentation of human neutrophils [Yabuki et al., 1999].

The most often cited protein in the literature as being critical to the process of segmentation in the neutrophils is the lamin B receptor (LBR) [Olins and Olins, 2004]. Some studies suggested that sufficient levels of this protein are required for non-ovoid nuclear shape [Hoffmann et al., 2007; Olins et al., 2008a]. In addition, Kasbecar [2012] found that sterol C-14 reductase activity of LBR is essential for neutrophil differentiation.

Cohen et al. [2008] found that loss of LBR modifies fibroblast nuclear morphology, changes the localization of other NE associated proteins, and the LBR is necessary for the morphological differentiation of granulocytes nuclei. In addition, mutations in LBR results in Pelguer–Huet anomaly (hypolobulation) and LBR mutations in mice result in ichthyosis (hypolobulated or kidney-shaped nuclei) [Olins et al., 2008a].

However, although this protein exerts great influence, the elevation of LBR content appears not be sufficient to promote nuclear lobulation [Hoffmann et al., 2007]. So, other factors may modulate LBR activity [Shultz et al., 2003]. Unfortunately, the mechanism by which LBR controls neutrophil nuclear shape and heterochromatin distribution remains largely speculative [Zwerger et al., 2008].

Nevertheless, Ma et al. [2007] found that the N-terminus of LBR is required for NE invagination and that N-terminal domain interacts with importin-β through Ran-GTPase regulation during mitosis of HeLa cells. They suggest that the LBR targeting of membrane to chromatin through importin-β contribute to the fusion of membrane vesicles and formation of NE. Although the neutrophil not perform mitosis, it is possible that protein machinery normally used during mitosis be used for induction of multi-lobulation during maturation process.

The LBR is associated with decondensing mitotic chromosomes during NE reformation [Zwerger et al., 2008] and increased levels of mitotic specific histone modifications such histone phosphorylation at serine 10 (H3(S10) p) are found in normal granulocytes [Olins et al., 2008a]. Furthermore, if we analyze the mitosis process we can find a similar moment to what would be a "bilobulization" before nuclear division, but more studies are required to support this suggestion. Again, Olins et al. [2008a] found low-to-negligible amounts of LBR in normal human granulocytes. This found again lead us to believe that the action of LBR in multi-lobulization may be more intense during the maturation and is more related to the acquisition than to the conservation of nuclear morphology.

Nuclear morphology may also be related to chromatin [Campbell et al., 1995]. Several authors considered the possibility that the spatial organization of chromosomes may contribute to nuclear morphology composition. However, Sanchez et al. [1997] conclude that chromosome position, although a necessary determinant of certain nuclear appendages does not play a role in nuclear segmentation because lobes within a neutrophil population vary in chromosome composition.

IMPORTANCE OF THE NUCLEUS IN THE FORMATION OF NEUTROPHIL EXTRACELLULAR TRAPS

In addition to undergo apoptosis or necrosis it has been discovered a new type of cell death applied to human neutrophils. This cell death is called NETosis that occurs in peculiar situations to aid the defense system, where the cell releases fibers or nets consisting basically of DNA, histones, and granule proteins. These are the neutrophil extracellular traps (NETs), death traps that settle in tissues, able to trap, and kill bacteria [Brinkmann et al., 2004].The NETs are released after specific neutrophil activation. The nuclear morphology will be modified, the nucleus loses its lobules and the chromatin decondenses. At the same time, occurs the disintegration of the granules. After that, the nuclear envelope disaggregates into vesicles and the nucleoplasm and cytoplasm mix to form a homogenous mass. Finally, apparently the cell contracts at certain points in the membrane and occur some ruptures that will permit the ejection of the NETs out of the cell [Brinkmann and Zychlinsky, 2012].

The downstream molecular mechanisms that induce NETs formation are not yet known. However, the literature already indicates some subjects with essential participation in this process such as reactive oxygen species and NADPH-oxidase complex [Brinkmann and Zychlinsky, 2007; Papayannopoulos et al., 2010]. Moreover, Wang et al. [2009] and Leshner et al. [2012] suggest that histone hypercitrullination (which is catalyzed by peptidylarginine deiminase 4 (PAD4)) mediates dramatic hetechromatin decondensation, chromatin unfolding, and finally neutrophil extracellular trap formation. In addition, Papayannopoulos et al. [2010] demonstrates that neutrophil elastase escapes from azurophilic granules and translocates to the nucleus to degrade specific histones during the cell activation. Finally, Farley et al. [2012] find that Serpin B1 restricts the NETs production in neutrophils.

CONCLUSION

The cell nucleus is critical for the occurrence of any eukaryotic cell differentiation process. Understanding the function of this organelle in each cell will bring us important data for better understanding of

the differentiation process. This review provides basic information about neutrophil nucleus structure, particular aspects about nuclear morphology, and the importance of this organelle in the neutrophil.

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