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Cardiac CD68+ and stabilin-1+ macrophages in wound healing following myocardial infarction: From experiment to clinic

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ABSTRACT

Myocardial infarction (MI) remains the leading cause of mortality and morbidity throughout the world. Macrophages are key innate immune cells that play a significant role in transition from the inflammatory to the regenerative phase during wound healing following MI. The scavenger receptor stabilin-1 is one of the most interesting macrophage biomarkers. This receptor contributes to wound healing, angiogenesis, and tissue remodeling. We suggested a research protocol using macrophage biomarkers to study the cellular basis of cardiac remodeling and healing in patients with acute MI. The purpose of the research was to translate experimental knowledge regarding macrophage subsets and their biomarkers in post-infarction myocardial regeneration into results observed in clinical settings. The study included 41 patients with fatal MI type 1. All patients were divided into four groups according to the timeline of MI histopathology. In addition to routine histopathological analysis, macrophage infiltration was assessed by immunohistochemistry. We used CD68 as a marker for the cells of the macrophage lineage and stabilin-1 as an M2-like macrophage biomarker. The number of CD68+ and stabilin-1+ macrophages in the infarct area increased and peaked in the regenerative phase and did not decrease in the late stage of MI. In the peri-infarct area, the number of CD68+ macrophages increased in the inflammatory phase, peaked during the reparative phase, and did not decrease in the late phase, while the number of stabilin-1+ macrophages increased in the regenerative phase and remained unchanged. Additionally, in the reparative phase, we observed increase in the number of CD68+ and stabilin-1+ macrophages in the non-infarct area. The research protocol suggested allowed us to translate experimental knowledge regarding macrophage subsets and their biomarkers in post-infarction myocardial regeneration into clinical data. Taken together, these results demonstrated biphasic cardiac macrophage response following acute MI somewhat similar to that in a murine model. The increase in stabilin-1+ macrophage infiltration noticed in the myocardium during the regenerative phase and the strong positive correlation between the number of these cells and timeline of MI histopathology enabled us to propose stabilin-1 as a diagnostic macrophage biomarker in myocardium wound healing in patients with acute MI.

1. Introduction

Myocardial infarction (MI) and consequent heart failure remain the leading cause of mortality and morbidity throughout the world (Montecucco et al., 2016; Ishii et al., 2008). Modern efficient acute care leads to reduction of acute infarct mortality but has contributed to an increase in the prevalence of heart failure. The necessity of better understanding, prevention, and treatment of heart failure resulted in exploration of new therapeutic strategies to repair the infarct heart (Christia and Frangogiannis, 2013; Karpov et al., 2005; Ryabov et al., 2006). Myocardial regeneration has become one of the most ambitious goals in prevention of adverse cardiac remodeling and consequent heart failure (Snyder et al., 2016).

Monocytes/macrophages, being key cells of the innate immune system, have become a subject of scientific interest due to their significant role in transition from the inflammatory to the regenerative

Abbreviations: IA, infarct area; MI, myocardial infarction; NIA, non-infarct area; PIA, peri-infarct area

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phase during wound healing following MI (Nahrendorf et al., 2007; Shiraishi et al., 2016; Nahrendorf et al., 2010). An experimental murine model of MI showed biphasic monocyte response in the myocardium (Nahrendorf et al., 2007). Pro-inflammatory M1 or Ly-6C^{high} macrophages dominated in the early phase of post-infarction cardiac remodeling (on days 1–4 after MI), whereas reparative M2 or Ly-6C^{low} macrophages dominated on days 4–10 during the resolution of inflammation. A similar biphasic peripheral blood monocyte response was demonstrated in patients with acute MI (Tsujioka et al., 2009). It seemed that monocyte/macrophage subsets mediated distinct biological functions, playing a dual role in wound healing and regeneration following MI. At the same time, the understanding of potential differences between human and mouse tissue macrophage responses to ischemic injury is needed, taking into consideration the influence of a large number of factors in clinical situations.

However, not only high heterogeneity of monocytes/macrophages has made these cells a subject of interest (Nahrendorf and Swirski, 2013; Murray and Wynn, 2011; Fujiu et al., 2014). Monocytes/macrophages have a remarkable plasticity that allows them to change their function and phenotype in response to microenvironmental signals (Gombozhapova et al., 2017; Troidl et al., 2009). Recent findings demonstrated the presence of numerous macrophage phenotypes in both homeostatic and pathological situations that did not show clear dichotomous states (Xue et al., 2014). The phenotype plasticity has been also observed during disease progression in an MI model (Nahrendorf and Swirski, 2013). Therefore, the dichotomous M1–M2 model that subdivided macrophages into a number of fixed states (M1, M2a, M2b and M2c) is not sufficient to completely describe macrophage activation and polarization. As a result, a multidimensional concept of macrophage ontogeny, activation, and function has been proposed (Ginhoux et al., 2016). According this concept, *in vitro* macrophages would be characterized by inducing signals, whereas *in vivo* macrophages would be characterized by multiple biomarkers. New evaluation of macrophage classification may influence understanding of the activation and characterization of these cells at the organ and tissue level not only in experiments but also in clinical conditions.

Molecular biomarkers of monocytes/macrophages identified to date have provided advanced diagnostic and predictive capabilities (Gratchev et al., 2012; Kzhyshkowska et al., 2016; Biswas and Mantovani, 2010; Murray et al., 2014). These molecular biomarkers allow characterizing different cell subpopulations and provide the foundation for clinical implementation of monocyte/macrophage-based diagnostic techniques and therapeutic approaches targeting these cells. One of the multifunctional and interesting macrophage biomarkers is the scavenger receptor stabilin-1 (Kzhyshkowska, 2010; Kzhyshkowska et al., 2006). This receptor is expressed by tissue macrophages and sinusoidal endothelial cells in homeostatic conditions. Furthermore, its expression is stimulated by chronic inflammation and tumorigenesis (David et al., 2012; Riabov et al., 2016; Mitrofanova et al., 2017). Macrophages, by expressing stabilin-1, mediate degradation of acetylated low-density lipoproteins and glycoprotein SPARC (secreted protein acidic and rich in cysteine), a universal regulator of wound healing, angiogenesis, and tissue remodeling (McCurdy et al., 2011). Studies investigating the role of stabilin-1 in cardiovascular diseases are not numerous. For instance, stabilin-1 expression was significantly elevated in monocytes in patients with familial hypercholesterolemia (Gratchev et al., 2013; Kzhyshkowska et al., 2012). In another study, a high number of stabilin-1 + macrophages was associated with larger areas of interstitial fibrosis in patients with chronic heart failure and left ventricular assist device support (Potapov et al., 2013). The expression and functional role of stabilin-1 in cardiac wound healing in patients with MI has not yet been investigated.

MI is a temporally and spatially dynamic process. Hence, it provides the opportunity to study macrophage phenotypes and their functions in wound healing and regeneration, as was done in a murine model. It was shown that M2-like macrophages contributed to wound healing. However, their exact role, dynamics, and phenotypes in the tissues *in vivo* and during pathological conditions in clinical situations remain unclear. In this study, we translated experimental knowledge regarding macrophage subsets and their biomarkers in wound healing following MI into clinical data. We suggested a protocol based on using macrophage biomarkers to investigate the cellular basis of cardiac remodeling in patients with MI.

2. Materials and methods

The study was approved by Biomedical Ethics Committee of the Cardiology Research Institute, Tomsk NRMC (protocol № 128). The study complied with the Declaration of Helsinki and was performed in accordance with Federal laws and regulations and institutional policies. The post-mortem examination was performed according to Order No. 354н (2013) issued by the Ministry of Healthcare of the Russian Federation. Informed consent was impossible and impracticable for the research, therefore the study was approved by a Local Ethics Review Board (protocol № 128). Thus, there was no contradiction to the Declaration of Helsinki (Informed Consent, point 32).

2.1. Clinical data analysis

Clinical and postmortem data from 41 medical histories and records of autopsies, performed in the Cardiology Research Institute (Tomsk, Russian Federation) in 2013–2014, were included in the study. The inclusion criterion was fatal MI type 1 (Taylor, 2012). Exclusion criteria were MI type 2–5, the presence of infectious complications (sepsis, pneumonia)/oncology diseases/valvular heart diseases, and cases when MI was not the primary cause of death.

2.2. Histopathological analysis

We used a biobank of tissue samples for analysis. Paraffin-embedded blocks of cardiac tissue were prepared for microtome sectioning followed by staining by hematoxylin and eosin. In each case, the tissue samples were re-examined histologically using light and polarized microscopy (Axio Imager M2 microscope, Zeiss). This approach allowed for identification of MI localization and its time of onset.

The first 24 h of MI were characterized by the absence of necrosis and the presence of prenecrotic changes such as myocyte hypereosinophilia, contraction bands, and intracellular myocytolysis (Mallory et al., 1939; Sommers and Jennings, 1964; Mitrofanova and Amineva, 2007). The following 24–72 h of MI were characterized by necrosis and predominantly polymorphonuclear leukocyte infiltration. An increased number of macrophages, phagocytosis of debris, and formation of granulation tissue were observed during days 4–10 (Mallory et al., 1939; Sommers and Jennings, 1964; Mitrofanova and Amineva, 2007). The signs of granulation tissue maturation (the presence of mature vessels, increase in number of fibroblasts and collagen fibers, and decrease in number of mononuclear cells) characterized 11–28 days of MI (Mallory et al., 1939; Sommers and Jennings, 1964; Mitrofanova and Amineva, 2007).

All cases were divided into four groups according to histopathological analysis (timeline of MI histopathology). Group 1 comprised patients who died during the first 24 h of MI; group 2 comprised patients who died within 24–72 h of MI; group 3 comprised patients who died on days 4–10; and group 4 comprised patients who died 11–28 days