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Plasma nuclear and mitochondrial DNA levels in acute myocardial infarction patients

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In this issue of *Coronary Artery Disease*, Wang et al. investigated the use of plasma DNA as a novel early biomarker for acute myocardial infarction (AMI) in patients undergoing a coronary catheterization for symptomatic heart disease. They discovered that, at the time of diagnostic catheterization, plasma levels of nuclear (n) and mitochondrial (mt) DNA were elevated in patients with AMI compared to patients without AMI, thus pointing to the prospect that analysis of plasma DNA could comprise an earlier and perhaps more cost-effective means of clinical laboratory detection than the other analyses currently employed. These observations also add some urgency to the need for an improved understanding of how DNA fragments are elaborated into the circulatory system and, perhaps of even greater significance, whether free DNA fragments function as mediators of the injury via their ability to activate resident and itinerant inflammatory and other tissue-specific effector cells [1, 2].

Levels of plasma mtDNA were approximately eight-fold higher than nDNA, but both decreased to the levels of the non-healthy controls by day 3. Although the authors suggested the increased levels of plasma DNA was secondary to cell death, there are reasons to suspect that mechanisms promoting release of mtDNA fragments might be cell type-specific, and not always linked to cell death per se. For example, stimulated eosinophils and dendritic cells both release mtDNA fragments into the extracellular environment in the absence of cell death [3-6]. In AMI, as well as the other disorders in which DNA fragment release into the extracellular space has been described, the cellular sources and mechanisms of release have yet to be completely defined and could be fruitful areas for further study.

The mechanisms triggering cellular export of DNA fragments are also unknown. In eosinophils, for example, mtDNA release has been described as a “catapult-like” process not involving conventional motor proteins [3]. Emerging evidence also suggests that oxidative

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Conflict of Interest: The authors declare no conflict of interest for this editorial. Of note, MNG has licensed a patent on fusion proteins targeting DNA repair enzymes to mitochondria. The mitochondrial-targeted DNA repair enzymes are currently under development for use in lung transplant, trauma/sepsis-related multiple organ dysfunction, and other forms of mtDNA DAMP-induced organ dysfunction.

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damage to mtDNA may play a critical role. In this regard, it has been known for some time that the mitochondrial genome is far more sensitive to oxidative damage than nuclear DNA [7, 8], an observation whose significance is underscored by involvement of reactive species of oxygen and nitrogen across the spectrum of pathologic processes associated with isolated and multiple organ dysfunction, including AMI [9, 10]. In direct support for a role for oxidative mtDNA damage in promoting release of extracellular mtDNA following AMI, our preliminary observations showed that pharmacologic enhancement of mtDNA repair in isolated rat lungs blocks bacteria-induced oxidative mtDNA damage and extracellular accumulation of mtDNA DAMPs [11].

An important question to emerge from the present study is whether the free DNA fragments mobilized in the setting of AMI contribute to ischemic myocardial damage. There are conflicting data that bear on this concept. First, in support for the postulated importance of mtDNA damage-induced mtDNA DAMP formation in AMI, Yang et al. found in a rat model of ischemia-reperfusion myocardial injury that intravenous administration of a fusion protein targeting the initial enzyme in base excision DNA repair, Ogg1, to mitochondrial reduced both mtDNA damage and infarct size [12]. Involvement of DNA DAMPs in this process was inferred by observations that, similar to enhancement of mtDNA repair, treatment of the animals with DNase1 to degrade circulating DNA also abrogated infarct size. Moreover, when isolated rat hearts were subjected to transient ischemia, infarct size was enlarged considerably by simultaneous exposure of the cardiac tissue to exogenous mtDNA fragments. Counter to the postulated importance of mtDNA damage and DAMPs in AMI, transgenic mice deficient in one or two key DNA glycosylases, either 8-oxoguanine DNA glycosylase or MutY glycosylase, fail to display exagerrations in either infarct size or cardiac function in comparison to wild type controls [13]. One plausible explanation for these divergent results is that, in the knockout mouse experiments, the DNA glycosylases were deficient in both the nuclear and mitochondrial compartments. Nuclear Ogg1 is known to play a role in transcriptional signaling [14, 15], including regulation of pro-inflammatory genes [16-19]. Obviously, nuclear Ogg1 (and MutY) deficiency could modulate the evolution of AMI in this animal model by mechanisms independent of mtDNA repair. In addition, given the fact that multiple DNA glyosylases are expressed in mammalian cells, there is the possibility of compensatory increases in expression or activities of other glycosylases in knockout animals.

In severely injured or septic human patients, circulating abundances of mtDNA fragments are associated with poor outcomes, including particularly multiple organ system failure [20-22]. In light of laboratory experiments demonstrating that administration of exogenous mtDNA DAMPs leads to widespread inflammation mediated by activation of TLR-9 receptors on innate immune and resident tissue effector cells [1, 2], it has been postulated that mobilization of mtDNA DAMPs by isolated tissue damage serves to propagate injury to distant organs thereby leading to multiple organ system dysfunction[1]. This concept leads to the question of why AMI, which is also accompanied by elevations in circulating mtDNA and nDNA fragments, is not generally linked to systemic inflammation and failure of non-cardiac organs by mechanisms not related to hemodynamic dysfunction. Of course, there are multiple explanations for this. The magnitude or persistence of the rise in circulating DNA evoked by the comparatively small amount of tissue damaged in AMI

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might not be adequate to trigger propagation of injury to non-cardiac sites. In this regard, our previous work in patients with severe trauma showed that individuals with similar injuries presented with either high plasma mtDNA that remained elevated until the patient developed MODS and death, or the patient presented with low plasma mtDNA levels which did not lead to MODS [22]. By contrast, Wang et al. found in patients with AMI that plasma mtDNA levels decreased over a three-day period to the baseline of the non-healthy controls. Another consideration is that predisposing factors which could augment sensitivity to mtDNA DAMPs, such as enhanced expression of TLR-9 [23], could be absent in the setting of AMI. Finally, the possibility also should be considered that there are indeed non-cardiac consequences of AMI that are DAMP mediated. In this context, literature now several decades old noted that in some AMI patients displayed pulmonary edema caused by enhanced vascular permeability rather than hydrostatic mechanisms [24-27]. Perhaps elevated circulating DAMPs triggered by ischemic cardiac damage contribute in subtle ways to the evolution of so-called “cardiogenic” pulmonary edema. Clearly, future studies will be required to address this possibility.

The provocative evidence provided in the current report by Wang et al suggesting that plasma DNA levels predict the occurrence of AMI should be pursued. Early, cost-effective laboratory determination of AMI would certainly have an impact on clinical management of this patient population. Among the various technical hurdles that needs to be overcome before this potential can be realized revolves around the fact that there is little standardization in the literature in terms of how mtDNA abundance is reported (i.e. relative difference, copy-number, ng/mL, etc.). For the field to advance, it might be beneficial for an expert panel to define methods for isolation and quantitation of mtDNA and nDNA values to guide future clinical studies of these novel outcome markers.

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