Revisiting p53 and its effectors in ischemic heart injury

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p53 is a tumor suppressor that enforces normal growth control and genomic stability. Its importance is reflected in the fact that acquired mutations in it or its upstream activators are found in all major human cancers [1]. p53 controls aberrant or inappropriate growth by acting as a transcription factor for a number of genes that promote apoptosis or growth arrest (Fig. 1). In addition, recent studies indicate that there are important non-transcriptional effects of p53 on apoptosis that involve its ability to suppress anti-apoptotic or activate pro-apoptotic Bcl-2 family members [2]. p53 expression or activity increases in cells in response to DNA damage and other forms of cell stress, including hypoxia [3], a major component of ischemic injury. Controversy over what role, if any, p53 plays in the response of the heart to ischemic injury dates back almost a decade. Three recent reports examine the role of p53 or well-characterized effectors of p53 in ischemia-induced injury. One, which is published in this issue of Cardiovascular Research, re-examines the role of p53 in ischemia-induced apoptosis, with a focus on the effects of p53 status on myocardial cell apoptosis and cardiac rupture [4].

1. p53 and cardiomyocyte cell death in culture

Following reports that p53 protein levels increase in non-myocyte cell lines in response to hypoxia [3] and that hypoxia could cause cell death of cultured cardiomyocytes [5], Long et al. [6] showed that hypoxia increases p53 expression in cardiomyocytes and that this increase occurs in connection with myocyte cell death. Although not all the data are in agreement regarding increased p53 expression in response to hypoxia [7], a recent study also reported increased p53 expression and cell death in cardiomyocytes exposed to hypoxia [8].

2. In vivo veritas

A persistent issue with all cell culture experiments is whether in vitro conditions can adequately recapitulate the complex series of events that occur in association with disease or a pathological situation. Animal models can often provide a relevant in vivo alternative for experimentation. Using the available p53 knockout mouse and looking at changes in apoptosis occurring 48 h after imposition of ischemia caused by coronary arterial ligation, Bialik et al. [9] showed that there was no difference in the amount of apoptosis (measured either by DNA laddering or TUNEL staining) between wild-type and knockout mice. Ex vivo studies on Langendorff perfused hearts taken from p53+/+ and p53−/− mice and subjected to 20 min of global ischemia (no flow) and 3 h of reperfusion also showed no difference in DNA laddering [7]. The results from these studies were very compelling when first published and essentially laid to rest the notion that p53 plays any role in the death of cardiomyocytes that occurs during and soon after an acute ischemic insult, at least in the mouse.

In this issue of Cardiovascular Research, the results of a study on the role of p53 in infarct-associated cardiac rupture are presented by Matsusaka et al. [4]. This study also used the wild-type and p53-deficient mice to examine the effects of p53 status on the incidence of death caused by cardiac rupture in response to coronary artery ligation. Ligation produced relatively large infarcts (~60%) and a substantial...
number of deaths attributable to cardiac rupture—8 out of 29 animals over the first 5 days post-ligation in wild-type (p53+/+) mice. Interestingly, p53+/-- mice exhibited a dramatic reduction in death (2 out of 28 animals) over this same period post-ligation, while p53/-/- mice showed no deaths. Importantly, p53 had effect on average infarct size, hemodynamic performance, tissue fibrosis, or matrix metalloproteinase (MMP) content. The lack of any difference in infarct size may not be surprising, since the primary determinant of cell loss during infarct is likely to be necrosis and p53 has only been linked to apoptosis and not necrosis. Likewise, no improvement in hemodynamic status over the period immediate after infarction would be expected without a change in infarct size. It is surprising, however, that there was no relationship between cardiac rupture and MMP content, since previous studies had established a connection between MMP2 expression/activity and cardiac rupture [10]. While MMP2 itself has not been directly linked to p53, p53 does control many genes involved in angiogenesis (see Fig. 1), a process that requires extensive extracellular matrix remodeling that, left unchecked, could compromise tissue integrity. In comparing the study by Matsusaka et al. [4] with the earlier report [10], it is important to point out that the earlier study reported no increase in MMP2 expression at 3 days post-ligation (the time point studied by Matsusaka et al. [4]), but did document an increase in expression and activation occurring sometime between days 3 and 7. In addition, zymography, the method used to measure MMP2 and MMP9 in both studies, detects total “activatable” amount and not in vivo activity. Increased activity is often correlated with the cleavage of pro-MMP2 to activated MMP2, but there is no evidence for such a product in the gels presented by Matsusaka et al. [4]. There remains, therefore, a significant amount of work that needs to be done to formally exclude an effect of p53 status on either extracellular matrix integrity or MMP activity/activation.

What did change in response to p53 status was the amount of observed cell death. Whether one (p53+/+) or both (p53/-/-) alleles were deleted, there was a marked reduction in myocardial cell death, as measured by TUNEL or DNA laddering. Unfortunately, only one time point (3 days post-ligation) was examined and this point did not correspond to any of the time points measured by Bialik et al. [9] or Webster et al. [7] in their studies using the p53/-/- mouse or hearts derived from these mice. In addition, it is not clear what percent of the cells scored as TUNEL-positive in the study by Matsusaka et al. [4] were, in fact, cardiomyocytes. The authors propose that it is the effect of p53 status on myocardial cell death that is responsible for the thinning of the ventricular wall to point of rupture. Inhibiting apoptosis independently of p53 status would help define the significance of these changes to cardiac rupture and rule out other effects of p53 deficiency. These results, of course, have significance beyond their potential connection to cardiac rupture because they are not what would necessarily have been predicted based on earlier studies that directly addressed the relationship between p53 status and cardiomyocyte cell death [7,9]. Does the nature of what controls apoptosis change with time post-ligation (3 days [4] vs. 4 days [9]), or does the fact that different-aged animals were used in the different studies matter? What is needed to resolve this possible conflict among these data is a more comprehensive study, confirming the results of what is reported in this issue [4], examining the effect of age on the response, and incorporating collection time points from both the study by Bialik et al. [9] and Matsusaka et al. [4].

### 3. Other recent studies

Two other recent reports focus on the contribution of well-described p53 effectors to ischemic injury in the heart.
or hypoxia/reoxygenation-induced injury in isolated neonatal cardiomyocytes [8,11] and are relevant to this discussion on p53 and the heart. A major effector of p53-mediated apoptosis is the BH3-only protein known as PUMA (p53-upregulated modifier of apoptosis) [12]. Toth et al. [11] examined the role of PUMA on infarct size, cell death, and functional hemodynamic parameters in an ex vivo model of ischemia/reperfusion of the mouse heart. Their results show that PUMA expression is up-regulated in response to ischemia and that infarct size was reduced by almost half in PUMA-deficient mice. The reduction in infarct size was accompanied by an almost complete return of hemodynamic parameters to normal. This effect of PUMA deficiency on infarct size (which is most likely due to an effect on necrosis) is in contrast to the lack of an effect of p53 deficiency on infarct size in the study by Matsusaka et al. [4] and suggests that up-regulation of PUMA expression may not be p53-dependent in the context of ischemic injury to the heart, although a formal test of this relationship in p53<sup>−/−</sup> mice would be relatively straightforward. If not p53, then what activates PUMA expression during ischemia? One tantalizing possibility would be the p53-related proteins, p73 and/or p63 [13]. These proteins transactivate many of the same genes (including PUMA) that p53 does, but are activated by different stimuli. Unfortunately, there has been no systematic study of p73 and p63 expression in the normal or ischemic heart, so that the evaluation of this possibility awaits new data.

In a separate study [8], the effect of mdm2 on ischemic injury to isolated cardiomyocytes and the intact heart was examined. Mdm2 is an E3 ubiquitin ligase that targets the p53 protein for proteosomal-mediated degradation. The study showed that cultured myocytes were less susceptible to hypoxia/reoxygenation injury when mdm2 was overexpressed, while inhibition of mdm2 caused cell death on its own and potentiated death in response to ischemia/reperfusion. These effects could be attributed to p53 destabilization and p53 stabilization, respectively, through changes in mdm2 expression/activity, although this was not directly tested. The protective effect of mdm2 was verified in isolated intact hearts from a hypomorphic mdm2 mouse line that exhibits reduced mdm2 expression. These hearts exhibited enhanced susceptibility to ischemia/reperfusion. Given what is currently known about mdm2, it is difficult to reconcile in a simple, linear model the fact that p53 deficiency has no effect on the response of the mouse heart ischemia/reperfusion injury [7,9] with the effect of reduced mdm2 expression promoting ischemia/reperfusion injury [8]. Again, a role for p73/p63 should be considered. Although p73/p63 are not substrates for the ubiquitin ligase activity of mdm2, mdm2 can bind to the transactivation domain of p73 [13], thereby suppressing its ability to transactivate apoptotic effectors such as PUMA (Fig. 1).

In conclusion, while these three studies may revive interest in a direct role for p53 in the response of the heart to ischemic injury (especially if the results of Matsusaka et al. [4] are reproduced and reconciled with what is already in the literature), it is more likely that they will help to refine our appreciation of the potential complexity of p53-related signaling pathways in the heart. It seems clear that the initial studies on p53 in isolated myocytes [6] may have been over-interpreted, and that conclusions based on enforced expression of p53 from that study and others may have been telling us more about pathways linked to p53 (e.g., p73/p63, PUMA, mdm2) than about the role of p53 itself in cardiac ischemic injury.

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References