CLINICAL IMPLICATIONS OF BASIC RESEARCH

Getting to the Heart of Proteomics

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Chen and colleagues[1] recently described mitochondrial aldehyde dehydrogenase type 2 (ALDH2) as a target in protecting the heart against ischemic insult. Their study provides insights into cardiovascular biology and may form the basis of a potential therapeutic approach to prevent or minimize myocardial ischemic injury in the clinical setting.

Cardiac cells have innate defense mechanisms against injury that results from prolonged disruption of blood flow as a consequence of a heart attack.[2-5] The presence of these mechanisms was originally revealed with the discovery that brief episodes of ischemia and reperfusion protect the heart against a subsequent prolonged ischemic insult. This process is known as ischemic preconditioning. Central to preconditioning is the activation of a signaling molecule, the epsilon form of protein kinase C (PKC-ε), which mobilizes an endogenous cardiac defense program against injury and thereby has an infarct-sparing effect.[3,5]

This discovery has spurred intensive research efforts to find pharmacologic agents that recapitulate the effects of ischemic preconditioning. Nitrates, adenosine agonists, and volatile anesthetics, which have been shown to be cardioprotective in the laboratory, have raised the hope of protecting patients from myocardial damage, particularly in clinical settings — such as in transplant and bypass surgery — in which ischemia can be anticipated. That said, translating experimental cardioprotective strategies remains a major challenge.

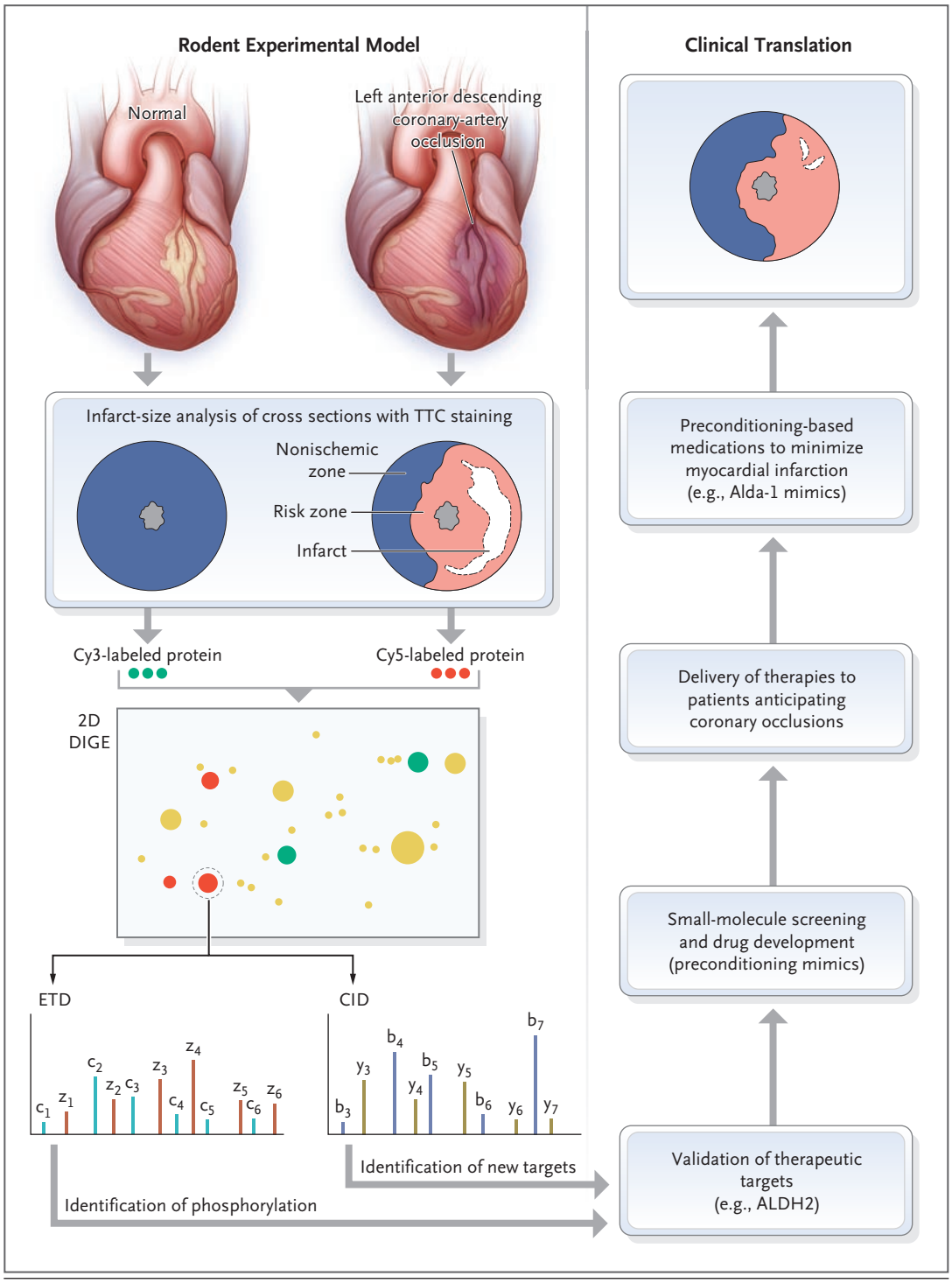
Investigations into the underlying cellular mechanisms of ischemic injury increasingly point toward a predominant role for mitochondria.[4] Mitochondria are not only the main energy producers of the cardiac cell but are also host to specific signaling pathways for biogenesis and programmed cell death. Myocardial ischemia leads to increased mitochondrial Ca^{2+} uptake, which in turn increases mitochondrial permeability, leading to the breakdown of mitochondrial respiration and ultimately cell death. During preconditioning, PKC-ε prevents this degenerative process[3,5] by phosphorylating molecules that trigger a cascade of events that ultimately preserve mitochondrial function.[2,4]

The mammalian mitochondrion contains more than 1500 different proteins, many of which have various types of post-translational modifications. Rather than evaluating single molecules one at a time, a proteomic analysis provides a broad-based portrait of the dynamic changes in mitochondrial proteomes during ischemia and reperfusion and offers an effective means of identifying protein targets that are relevant to disease.

The study by Chen et al. combines the power of proteomics with a classic animal model of myocardial infarction (Fig. 1). The authors report that phosphorylation of mitochondrial ALDH2 in response to protective stimuli (such as PKC-ε acti-

Figure 1 (facing page). The Power of Proteomics.

Using a proteomic approach, Chen et al.[1] recently analyzed experimental models of heart injury. They extracted protein from the hearts of laboratory animals that had undergone sham operations (sham hearts) or had ischemic injury (injured hearts) and then labeled the proteins with fluorescent dyes (e.g., Cy3 [green] and Cy5 [red]). They then mixed the samples and resolved the proteins with the use of triphenyltetrazolium chloride (TTC) staining on a two-dimensional gel according to isoelectric point and molecular weight (e.g., two-dimensional differential in-gel electrophoresis [2D DIGE]). Yellow circles indicate protein spots that are identical in the sham and injured hearts, green circles indicate protein spots that are more abundant in the sham hearts, and red circles indicate protein spots that are more abundant in the injured hearts. Protein spots of interest were excised from the gel, and the ions (c, z, b, and y) were analyzed by means of liquid chromatography–mass spectrometry. Mass spectrometers set for collision-induced dissociation (CID) are preferable for protein identification, whereas those set for electron-transfer dissociation (ETD) are preferable for the identification of phosphorylation sites. Target candidate proteins such as aldehyde dehydrogenase type 2 (ALDH2) are then validated and screened for the development of potential therapeutic agents.



vation or ethanol treatment) increased the enzyme's activity and reduced the size of a myocardial infarct in a rodent model of myocardial ischemic injury. Next, they identified Alda-1 as a specific ALDH2 agonist that activated different isoforms of ALDH2, including that encoded by *ALDH2**2, a common allele in a majority of populations in East Asia. When administered before the development of ischemia in rodents with global ischemic injury, Alda-1 reduced the infarct

size, thereby acting as a preconditioning mimetic. Activation of ALDH2 by Alda-1 was dependent on mitochondrial PKC-ε.

The proteomic approach used by Chen et al. to help translate insights from studies of preconditioning to the clinical arena circumvented preconceived notions about which molecules are involved and enabled the identification of a key molecular target of preconditioning. By coupling this approach with whole-animal physiological studies and small-molecule screening, they have provided a convincing and new mechanism with potential clinical relevance. Although substantive further research is clearly necessary, ALDH2 activators may at some point protect patients against myocardial infarction. Even limited restoration of ALDH2 activity by Alda-1 may be relevant to the prevention of myocardial infarction in persons who carry the *ALDH2**2* allele.

No potential conflict of interest relevant to this article was reported.

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