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Resource

Functional Interplay between Caspase Cleavage and Phosphorylation Sculpts the Apoptotic Proteome

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SUMMARY

Caspase proteases are principal mediators of apoptosis, where they cleave hundreds of proteins. Phosphorylation also plays an important role in apoptosis, although the extent to which proteolytic and phosphorylation pathways crosstalk during programmed cell death remains poorly understood. Using a quantitative proteomic platform that integrates phosphorylation sites into the topographical maps of proteins, we identify a cohort of over 500 apoptosis-specific phosphorylation events and show that they are enriched on cleaved proteins and clustered around sites of caspase proteolysis. We find that caspase cleavage can expose new sites for phosphorylation, and, conversely, that phosphorylation at the +3 position of cleavage sites can directly promote substrate proteolysis by caspase-8. This study provides a global portrait of the apoptotic phosphoproteome, revealing heretofore unrecognized forms of functional crosstalk between phosphorylation and caspase proteolytic pathways that lead to enhanced rates of protein cleavage and the unveiling of new sites for phosphorylation.

INTRODUCTION

Proteolysis and phosphorylation are two of the most pervasive forms of protein posttranslational modification, playing essential roles in the majority of (patho)physiological processes, including tissue development, cancer, and cell death (Kurokawa and Kornbluth, 2009; López-Otín and Hunter, 2010). Apoptosis, or programmed cell death, is orchestrated by a family of cysteine proteases called caspases, which cleave their protein substrates after aspartic acid residues (Crawford and Wells, 2011; Fuentes-Prior and Salvesen, 2004; Thornberry and Lazebnik, 1998). Recent advances in global protease substrate identification technologies have generated a large inventory of proteins that are cleaved by caspases during apoptosis, demonstrating that as much as 5% of the proteome is subject to caspase-mediated proteolysis (Arntzen and Thiede, 2011; Crawford and Wells, 2011).

Protein kinases are prominently represented among caspase substrates and, in some cases, cleavage activates these kinases so that they can perform important functions in apoptosis (Kurokawa and Kornbluth, 2009). Caspase-mediated activation of Rho-associated kinase 1 (ROCK1), for instance, promotes the characteristic membrane blebbing associated with apoptosis (Coleman et al., 2001). Kinases can also be inactivated by caspase-mediated cleavage to block their activity during apoptosis (Kurokawa and Kornbluth, 2009). The crosstalk between caspases and kinases also includes the phosphorylation of caspases to either enhance or suppress their activity (Kurokawa and Kornbluth, 2009). Likewise, the phosphorylation of some caspase substrates, notably BID phosphorylation on Thr59 (which is the P2 residue of the caspase-8 cleavage site) blocks caspase cleavage (Degli Esposti et al., 2003). These findings suggest that caspase and kinase pathways interact in intricate ways to influence the balance between cell survival and death (Janes et al., 2005). Nonetheless, whether a more global relationship between proteolysis and phosphorylation exists in apoptosis has not been investigated.

We recently introduced a proteomic method termed PROTOMAP (short for Protein Topography and Migration Analysis Platform) that can be used to characterize proteolytic events in cells by detecting shifts in protein migration through a combination of SDS-PAGE and mass spectrometry (MS)-based proteomics (Dix et al., 2008). Using this approach, we identified over 250 cleaved proteins in apoptotic cells, including 170 proteins that were not previously known to be cleaved by caspases. In the current study, we sought to create an advanced, quantitative version of PROTOMAP that enables simultaneous analysis of proteolytic and phosphorylation processes in cells, such that phosphorylation sites could be directly integrated into the topographical maps of cleaved proteins during apoptosis. We applied this method to study the intrinsic apoptotic cascade in Jurkat T cells, resulting in the identification of more than 700 cleaved proteins and 5,000 sites of phosphorylation. The integration of these global data sets revealed that phosphorylation events are enriched on cleaved proteins and

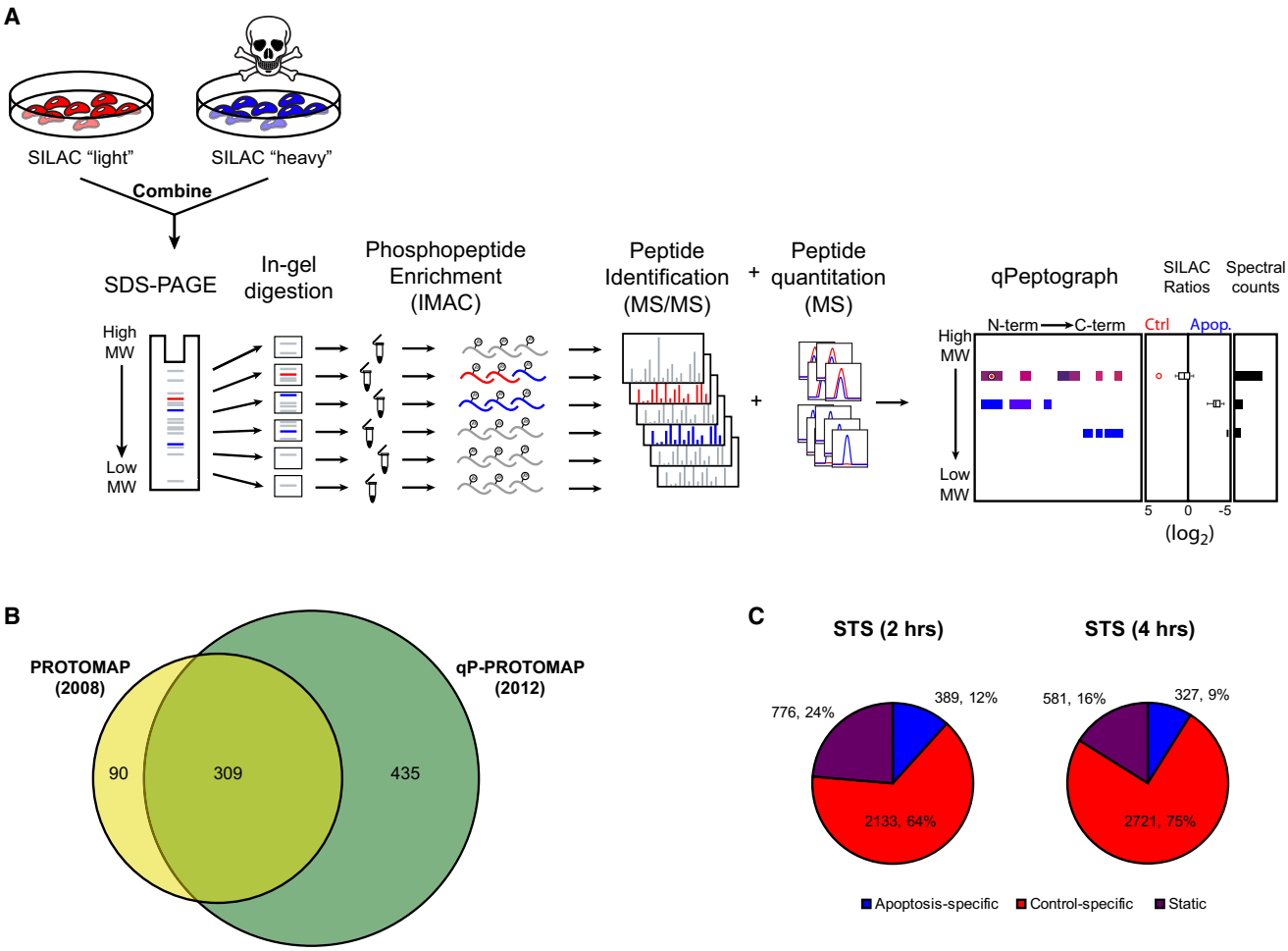

Figure 1. Quantitative Profiling of Phosphorylation and Proteolytic Pathways in Apoptosis by qP-PROTOMAP

(A) General features of qP-PROTOMAP method as described in the main text. Peptides are colored red and blue to represent signals detected in healthy control (light) and apoptotic cells (heavy), respectively.

(B) Number of cleaved proteins detected using the original PROTOMAP method (Dix et al., 2008) versus qP-PROTOMAP as described in this study. See Table S1 for peptographs of cleaved proteins identified by qP-PROTOMAP.

(C) Distribution of phosphorylation events identified in control and apoptotic cells by qP-PROTOMAP. Phosphorylation events were designated "control specific" or "apoptosis specific" if they showed light/heavy SILAC ratios of >2 or <0.5, respectively (corresponding to $\log_2$ values of 1 or −1). Phosphorylation events displaying light/heavy ratios between these values were designated as "static."

See also Table S2.

are clustered around sites of caspase cleavage. We further identified a cohort of previously unreported phosphorylation sites that were specific to apoptotic cells, suggesting the existence of a cell-death-related phosphorylation network. We show using activity-based proteomic methods that at least a part of this network is driven by caspase-mediated activation of DNA-dependent protein kinase (DNA-PK) at early stages during the time course of apoptosis. Finally, we interrogated the functional relationship between proteolysis and phosphorylation, uncovering multiple forms of crosstalk that include the caspase processing of proteins to expose new sites for phosphorylation and the phosphorylation of proteins at the +3 (P3) position of caspase recognition sequences to dramatically enhance proteolysis by caspase-8.

RESULTS

Quantitative Proteomic Analysis of Phosphorylation and Proteolysis by qP-PROTOMAP

The proteomic measurement of dynamic posttranslational modifications, like phosphorylation, requires quantification of individual peptides, and we therefore sought to combine PROTOMAP with stable isotopic labeling methods (SILAC; Ong et al., 2002) for this purpose. We also needed to incorporate a phosphopeptide enrichment step without sacrificing the protein size and topography information provided by the SDS-PAGE step of the original PROTOMAP method. The workflow for the resulting quantitative phospho-PROTOMAP (or qP-PROTOMAP) platform was therefore as follows (Figure 1A): Control and