432b Microdevice for High Throughput Analysis of Cross-Talk between Signaling Pathways: Application to Heat Shock and Apoptosis Responses in Liver Cells

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Heat shock (HS) response is a protective mechanism for cells to prime themselves for further lethal stress. Heat up-regulated HSP expression reduced the apoptosis from TNF stimulation. However, vector-mediated overexpression of HSP70 failed to provide the protection function, and on the contrary, it sensitized cells to TNF induced apoptosis. We proposed a comprehensive correlation study between the dynamic change of HSP expression and levels of apoptosis induced by TNF after HS. To do this, mild HS at 42^{o}C for 2 hours followed by varied recovery times was used to produce different amount of HSPs within its physiological range. TNF was chosen to induce apoptosis in H35 hepatoma cells at the different recovery times after HS. Both conventional biochemistry methods with H35 cells and a high throughput microfabricated fluidic device with H35 NFêB reporter monoclonal cells were used in this study. An optimal HS protection window against TNF induced apoptosis was illustrated distinguishably, and most likely HS sensitized cells to TNF stress in 2 hr recovery window. The correlation study between apoptosis and HSP expression kinetics demonstrated that physiologically high level of HSP70 and HSP27 as well as proper timing between HS and TNF stress are critical to have the optimal protection window. The results for the microfluidic duration device indicated that NFκB cell survival pathway might not be involved in the production of the HS protection window, while NFκB activity was definitely suppressed in apoptosis sensitive window. The results from this study may be used as a molecular basis for the further investigation that uses HS as a clinical approach to target tissue survival or death, such as enhancing cancer cell death and protecting nonmalignant cells from apoptosis in anticancer therapy.