Abstract

Mutations in the small heat shock protein (sHSP) αB-crystallins cause a range of human diseases including dilated cardiomyopathy (DCM), desmin-related myopathy (DRM) and congenital cataracts. DRM was the first discovered associated with desmin mutations which can affect themselves and closely interacted proteins that typically tending to incomplete assembly of desmin and formation of electro-dense granulofilamentous materials. These diseases involve the disruption of the intermediate filament (IF) cytoskeleton and thus identified intermediate filaments as important physiological targets of sHSPs. Recently, a missense mutation Gly154Ser (G154S) in αB-crystallin was reported to be associated with a late-onset distal vacuolar myopathy without cardiac or respiratory dysfunction and cataracts. This mutation affects a highly conserved amino acid residue among the αB-crystallin in mammals and has been identified earlier in patients with isolated cardiomyopathy. In this study, the effects of G154S mutation on αB-crystallin's ability to interact with desmin IFs was investigated in details using a combination of biochemical, molecular and cell biological approaches. Cosedimentation assay showed that the G154S mutation increased the binding of αB-crystallin to assembled desmin IFs. Transmission electron microscopy confirmed that the G154S αB-crystallin particles decorated the assembled desmin filaments. Transient transfection studies revealed that the expression of G154S αB-crystallin did not affect its distribution but accomplishment of slightly decreased of solubility in C2C12 cell fraction study compared to the wild type αB-crystallin. This is in contrast to the R120G mutation reported in desmin-related myopathy, where this mutation affected the solubility of αB-crystallin and promoted its interaction with desmin filament leading to intracellular aggregates formation in transiently transfected cells. When transfected into a range of cell lines, both R120G and G154S αB-crystallin mutants, but not the wild type protein, increased the phosphorylation of αB-crystallin at Ser^{59} site. Taken together, these data suggest that the G154S mutation may be involved in the pathogenesis of myopathy through a mechanism that is different from the R120G mutation found in DRM.

中文摘要

小型熱休克蛋白：αB水晶體蛋白的突變是造成多種疾病的原因，例如：擴張型心肌病變、肌間線蛋白肌病變，以及先天性的白內障。其中，肌間線蛋白肌病變最早被發現其致病因素直接和肌間線蛋白的突變有關，此突變蛋白不僅改變了本身蛋白質的結構更牽連與它有密切結合關係的蛋白質，進而造成電子密度高的顆粒纖維狀物質的形成。此疾病與中間絲蛋白的細胞骨架瓦解有絕對的相關,由此可知小型熱休克蛋白對於中間絲蛋白具有重要的生理意義。小型熱休克蛋白被認為扮演一個伴隨蛋白的角色，可以和不正常折疊的蛋白質結合並避免形成不正常的蛋白質結構，進而讓細胞遠離逆境壓力。一個位於αB水晶體蛋白上的第154個胺基酸上的誤義突變：由甘胺酸突變成絲胺酸，最近被檢驗出此突變和晚發性的末梢液泡肌病變有關，而與一些典型的心肌病變或是呼吸缺陷還有白內障這些疾病無關。此一突變位於哺乳動物高度保留性的αB水晶體蛋白基因序列中，並更早就被發現於一個罹患單純心肌症的病人檢體中。這項研究藉由生物化學以及分子與細胞生物的方式去探討G154S-αB水晶體蛋白對於肌間線蛋白的交互作用的能力是否因為突變而造成改變。共沉降實驗分析顯示出G154S-αB水晶體蛋白增加了與肌間線蛋白結合的能力。利用穿透式電子顯微鏡觀察此兩種蛋白質的交互作用，也的確出現了G154S-αB水晶體蛋白的顆粒蛋白攀附在肌間線蛋白所形成的中間絲纖維上的現象。而在短暫型DNA轉染細胞系統的研究項目中，實驗數據顯示G154S-αB水晶體蛋白並不會因為突變了而影響它在人類乳癌細胞、老鼠肌原母細胞、以及倉鼠腎細胞裡的分佈，但是在C2C12的細胞比起正常的水晶體蛋白會降低了些微的溶解度。R120G-水晶體蛋白被指出同樣也會造成肌間線蛋白肌病變，藉此可對照R120G-水晶體蛋白在短暫型DNA 轉染細胞系統所引起的，明顯地促進與肌間線蛋白的結合能力、蛋白質溶解度的改變，以及導致形成細胞內不正常蛋白堆積物。另一方面，在R120G-水晶體蛋白和G154S-水晶體蛋白的第59號的絲胺酸上都出現了特殊地磷酸化的現象。綜合以上的研究結果，G154S-水晶體蛋白的致病機制可能與導致肌間線蛋白肌病變的R120G-水晶體蛋白不盡相同。