E105

Production and Characterization of Monoclonal Antibodies against the 90-kDa Heat Shock Protein

공희정*, 김홍로, 김정락[1], 강호성, 김한도
부산대학교 자연과학대학 분자생물학과, [1]인제대학교 자연과학대학 생물학과

The 90kDa-heat shock protein (HSP90) is one of the major ubiquitous heat shock proteins induced by a variety of cellular stresses. HSP90 is highly conserved during evolution and constitutively synthesized even under nonstressed conditions. HSP90 has been found in association with several regulatory and structural proteins such as protein kinases and steroid hormone receptors. In the present study, to facilitate its biochemical characterization, HSP90 was purified from chick muscle by sequential column chromatography steps including DEAE-cellulose, hydroxyapatite, and Sephacryl S-300 gel filtration and monoclonal antibodies specific to HSP90 were produced by the murine hybridoma technique. We report the production of 4 postive hybidoma clones, named as A204, C112, C302 and C410. Among these MoAbs, C112 strongly recognized chick HSP90 in Western blot and native immunoprecipitation. In addition, C112 showed the cross-reactivities against HSP90 from human, rabbit, mouse, fish and chick but not from Drosophila and *E. coli*.

E106

Inwardly Rectifying K^+ Channels in Developing Muscle Cells in Culture

박 재 용,* 신 기 순, 강 만 식
서울대학교 분자생물학과

We examined the developmental changes of inwardly rectifying K^+ channels and resting membrane potential in cultured chick muscle cells. The gradual increase of resting membrane potential during myogenesis was observed and inwardly rectifying K^+ currents were apparent from the aligned myoblasts which were just prior to fusion and increased thereafter. Inwardly rectifying K^+ channels in aligned myoblasts had single channel conductance of 31 pS measured in cell attached patch with 140 mM K^+ in the pipet, and the unitary I-V relations rectified. Myoblasts devoid of inwardly rectifying K^+ channels had low resting K^+ permeability, while myoblasts that posses inwardly rectifying K^+ channels had high resting K^+ permeability. Anomalous hyperpolarizations with increased extracellular K^+ concentration were observed in some myoblasts, due to the increased conductance of inwardly rectifying K^+ channels. Application of 0.1 mM Ba^{2+} to the bath abolished dramatically the inwardly rectifying K^+ currents but not the outwardly rectifying K^+ currents, with the resting membrane potential depolarized. We conclude that the inwardly rectifying K^+ channels that were expressed from the aligned stage of myoblasts may be responsible for setting the resting membrane potential during myogenesis.