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α-Crystallin localizes to the leading edges of migrating lens epithelial cells

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Abstract

α-crystallin (αA and αB) is a major lens protein, which belongs to the small heat-shock family of proteins and binds to various cytoskeletal proteins including actin, vimentin and desmin. In this study, we investigated the cellular localization of αA and αB-crystallins in migrating epithelial cells isolated from porcine lens. Immunofluorescence localization and confocal imaging of αB-crystallin in confluent and in migrating subconfluent cell cultures revealed a distinct pattern of subcellular distribution. While αB-crystallin localization was predominantly cytoplasmic in confluent cultures, it was strongly localized to the leading edges of cell membrane or the lamellipodia in migrating cells. In accordance with this pattern, we found abundant levels of αB-crystallin in membrane fractions compared to cytosolic and nuclear fractions in migrating lens epithelial cells. αA-crystallin, which has 60% sequence identity to αB-crystallin, also exhibited a distribution profile localizing to the leading edge of the cell membrane in migrating lens epithelial cells. Localization of αB-crystallin to the lamellipodia appears to be dependent on phosphorylation of residue serine-59. An inhibitor of p38 MAP kinase (SB202190), but not the ERK kinase inhibitor PD98059, was found to diminish localization of αB-crystallin to the lamellipodia, and this effect was found to be associated with reduced levels of Serine-59 phosphorylated αB-crystallin in SB202190-treated migrating lens epithelial cells. αB-crystallin localization to the lamellipodia was also altered by the treatment with RGD (Arg-Ala-Asp) peptide, dominant negative N17 Rac1 GTPase, cytochalasin D and Src kinase inhibitor (PP2), but not by the Rho kinase inhibitor Y-27632 or the myosin II inhibitor, blebbistatin. Additionally, in migrating lens epithelial cells, αB-crystallin exhibited a clear co-localization with the actin meshwork, β-catenin, WAVE-1, a promoter of actin nucleation, Abi-2, a component of WAVE-1 protein complex and Arp3, a protein of the actin nucleation complex, suggesting potential interactions between αB-crystallin and regulatory proteins involved in actin dynamics and cell adhesion. This is the first report demonstrating specific localization of αA and αB-crystallins to the lamellipodia in migrating lens epithelial cells and our findings indicate a potential role for α-crystallin in actin dynamics during cell migration.

Keywords: α-crystallin; Small heat shock protein; Lamellipodia; Migration; p38-MAPK and Actin dynamics

Introduction

α-Crystallin is a major lens protein which belongs to the small heat-shock family of proteins (sHSPs) and the two α-crystallins, αA- and αB-crystallins, exhibit an approximately 60% sequence identity and have many properties in common [1,2]. Both proteins exhibit striking sequence similarity with the sHSPs [3–5]. Both αA and αB-crystallins are expressed in a number of extralenticular tissues [6]. αB-crystallin in particular, is expressed at high levels in the heart, striated muscle, kidney and brain, and in several types of cells [2,7]. αB-crystallin localization is thought to be predominantly cytoplasmic, but recent studies have also documented a nuclear and centrosomal localization [8–10]. The expression of αB-crystallin is upregulated in response to several different stress factors, and increased levels of αB-crystallin have been found in various neurodegenerative diseases and in tumors [2,5,11]. αA and αB-crystallins are phosphorylated by mitogen-activated protein kinase-activated protein kinases [4,7,12], and both proteins are known to exhibit anti-apoptotic response against various stresses [13,14]. The α-crystallins are also

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known to act as molecular chaperones by virtue of their ability to prevent stress-induced aggregation of proteins in vitro [15]. Recently, αB-crystallin has also been reported to be involved in Golgi reorganization during the cell cycle [16].

In vitro αB-crystallin interacts with various cellular cytoskeletal components including actin based microfilaments, microtubules and intermediate filaments, and is therefore proposed to be involved in stress-induced assembly and disassembly of these structures [7,17–21]. Gene targeting studies of αB and αA-crystallins have been demonstrated to result in abnormal skeletal muscle phenotype and cataract formation, respectively, in mice [22]. A missense mutation in the αB-crystallin gene causes a desmin-related myopathy and cataract in humans [23].

αB-crystallin shares several characteristics with Hsp 27 [7,20]. Both proteins also bind to cytoskeletal proteins, and are phosphorylated by p38 MAP Kinase in response to stress factors [4,20,24]. Interestingly, Hsp27 appears to participate in cell migration in a phosphorylation-dependent manner [24,25] and is thought to regulate actin dynamics, focal adhesion formation and contractile function of SMC [26]. In contrast, very little is currently understood regarding the cellular function of αB-crystallin at large, particularly as it relates to a possible functional role in cell migration, which is a fundamental cellular process essential for embryonic development, wound healing, immune responses and development of tissues [27,28]. Given the shared properties between Hsp27 and αB-crystallin, and the ability of αB-crystallin to interact with elements of the cellular cytoskeleton, we sought to understand if αB-crystallin plays a role in the processes of lamellipodia formation.

In this study, using primary epithelial cells isolated from porcine lens, we determined the localization of αA and αB-crystallins in different cellular compartments by employing immunofluorescence detection and documenting the results using confocal microscopy. Our data reveal that in migrating lens epithelial cells, αB-crystallin localizes predominantly to the leading edges or the lamellipodia and that this localization appears to be dependent on p38 MAPK regulated phosphorylation of αB-crystallin. Additionally, we demonstrate that αB-crystallin localization to the lamellipodia depends on the activities of integrin, Rac GTPase and Src proteins.

Materials and methods

Reagents and antibodies

Affinity purified anti-αB-crystallin and αA-crystallin rabbit polyclonal antibodies raised against human recombinant proteins were obtained from Joseph Horwitz, Jules Stein Eye Institute, UCLA. Anti-phospho-αB-crystallin (Ser 59) rabbit polyclonal antibody was procured from Affinity Bioreagents (Golden, CO). Anti-WAVE-1 mouse monoclonal and anti-Abi-2 rabbit polyclonal antibodies were provided by Ann Marie Pendergast, Department of Pharmacology and Cancer Biology, Duke University School of Medicine (Durham, NC); Anti-Arp2/3 polyclonal antibodies were received from Matthew Welch, Department of Molecular and Cell Biology, University of California (Berkeley, CA). Anti-β-catenin monoclonal antibody was purchased from Zymed Laboratories Inc (San Francisco, CA). Anti-βB2, anti-γ-crystallin and anti-aquaporin-0 rabbit polyclonal antibodies were provided by Samuel Zigler from the National Eye Institute, NIH (Bethesda, MD). Adenoviral vectors, expressing the dominant negative Rac1 (N17) GTPase and the β-galactosidase reporter gene were provided by Toren Finkel from the National Heart Lung and Blood Institute, NIH, Bethesda, MD. FITC (Fluorescein isothiocyanate)-Phalloidin, TRITC (Tetramethylrhodamine isothiocyanate)-conjugated secondary antibodies, anti-ZO-1 antibody, gelatin type B, cytochalasin D, X-gal (5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside), DAPI (4,6-diamidino-2-phenylindol) and collagenase IV were from Sigma-Aldrich (St. Louis, MO). Inhibitors of ERK kinase (extracellular signal-regulated kinase) (PD98059), p38 MAP kinase (SB202190), Myosin II inhibitor (blebbistatin) and Src kinase (PP2) were procured from Calbiochem, San Diego, CA. RGD (Gly-Arg-Ala-Asp-Ser-Pro) and RGE (Gly-Arg-Ala-Glu-Ser-Pro) peptides were purchased from Gibco BRL Lifetechnology, Gaithersburg, MD and Rho-kinase inhibitor (Y27632) was provided by Welfide Corporation, Japan. The human lens epithelial cell line SRA-04, was provided by V.N. Reddy from Kellogg Eye Center, Michigan University School of Medicine, Ann Arbor, MI.

Lens epithelial cell culture

Primary lens epithelial cell (LEC) cultures developed from lenses extracted from freshly obtained enucleated porcine eyes were used in this study. Briefly, extracted lenses (free of any adherent tissues) were rinsed with medium 199 (Gibco BRL), prior to careful removal of the lens capsules. Using a pair of tying forceps, the outer cortical layer (approximately 2 mm thickness), together with its attached lens epithelium, was carefully peeled out. The outer cortical lens tissue was then minced and digested in medium 199 containing 1.5 mg/ml collagenase IV and 0.2 mg/ml porcine albumin at 37°C for 90 min. At the end of the digestion, the contents were centrifuged (2500 RPM) for 10 min at 10°C, and the cell pellet was suspended in Dulbecco's modified Eagle medium (DMEM) containing 20% fetal bovine serum (FBS), penicillin (100 Units/ml), streptomycin (100 μg/ml), and gentamicin (20 μg/ml), then plated on plastic Petri plates coated with 2% gelatin. Cell cultures were grown at 37°C, and under 5% $C0_2$. Primary LEC cultures generated using this procedure (second and third passages) was used throughout this study. Mouse (C57B6 strain) primary LEC and porcine trabecular mesh-