

POSTER PRESENTATION

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# cGMP-dependent protein kinase I $\beta$ phosphorylates and regulates the function of the actin/myosin-associated protein caldesmon

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## Background

The type I cGMP-dependent protein kinases (PKGI $\alpha$  and PKGI $\beta$ ) are splice variants that differ in their first ~100 amino acids, giving each isoform unique dimerization and autoinhibitory domains. The unique coiled-coil dimerization domains mediate isoform specific protein-protein interactions, and we have previously identified the amino acids that are important in mediating the interaction between PKGI $\beta$  and its two known interaction partners, TFII-I and IRAG [1].

## Results

Using wild-type and mutant PKGI $\beta$  D/D domains as affinity probes in a proteomic screen and we identified the actin/myosin associated protein caldesmon as a PKGI $\beta$  specific interacting protein [2]. Using immunofluorescent staining, we found that PKGI $\beta$  and CaD colocalized with F-actin at lamellipodial structures at the edge of MDA-MB-231 cells. We found that PKGI phosphorylated CaD in a species- and isoform-specific manner. Human type 5 caldesmon was phosphorylated on serine 12 by PKGI $\beta$  *in vitro* and in intact cells. Phosphorylation on serine 12 or a phospho-mimetic S12E mutation significantly reduced the interaction between CaD and myosin IIA. We found that siRNA mediated caldesmon depletion increases the migration of MDA-MB-231 cells, and that reconstitution with wild-type or phospho-deficient S12A caldesmon slowed migration. In contrast, migration was not slowed by reconstitution with caldesmon containing an S12E mutation. We also found that PKG activation leads to indirect phosphorylation of mouse and human CaD in 293T and MDA-MB-231 cells. The indirect phosphorylation seen

in 293T cells is accompanied by a shift in the apparent molecular mass of CaD during SDS-PAGE. The observed migratory shift is similar to that previously seen when purified platelet CaD was directly phosphorylated *in vitro* by PKA [3]. Indirect phosphorylation of CaD in MDA-MB-231 did not cause a migratory shift.

## Conclusion

Since serine 12 is not conserved in mouse or rat, our results indicate that PKGI $\beta$  regulates caldesmon in a species-specific manner. While the PKGI-mediated indirect phosphorylation site(s) have not been determined, our preliminary data suggests that PKGI could potentially regulate all CaD isoforms, albeit in a cell type and species specific manner.

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## References

1. Casteel DE, Boss GR, Pilz RB: Identification of the interface between cGMP-dependent protein kinase Ibeta and its interaction partners TFII-I and IRAG reveals a common interaction motif. *J Biol Chem* 2005, **280**:38211-38218.
2. Schwappacher R, Rangaswami H, Su-Yuo J, Hassad A, Spittler R, Casteel DE: cGMP-dependent protein kinase Ibeta regulates breast cancer cell migration and invasion via a novel interaction with the Actin/Myosin-associated protein caldesmon. *J Cell Sci* 2013, **126**:1626-1636.
3. Hettasch JM, Sellers JR: Caldesmon phosphorylation in intact human platelets by cAMP-dependent protein kinase and protein kinase C. *J Biol Chem* 1991, **266**:11876-11881.

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