Conformation of Loop 2 at motor domain determines the activity of Nonmuscle Myosin IIs

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Nonmuscle myosin II (NMIIs) are cytoskeletal actin binding motor proteins that can act as mechanical coordinator in various cellular processes such as migration, division and mechano-adaptation. NMIIs at actomyosin (AM) unit can hydrolyze ATP and pull actin filaments and hence can generate forces in the pN range which are required for executing those cellular processes. We have previously documented that alternative splicing at loop2 region alters NMII activity, and consequently, AM function. Interestingly, splicing at loop2 of NMIIA renders constitutively inactive whereas same modification in NM IIC or IIB produces an inactive mechanoenzyme. Recently, mutation in one of the paralogs, NMIIC, have been detected in cancer, but their roles in cancer progression remain elusive. Here, we characterize several point mutations in the NMIIC identified in gingiva-buccal oral squamous cell carcinoma, which were distributed across the N-terminal motor (head) domain (Glu291Gln, Asp597Tyr, Ser655Arg and Gln890Lys) and the C-terminal coiled-coil (tail) domain (Glu1733Gln, Glu1797Asp). Among these, the Ser655Arg at loop 2 of the motor domain enhanced actin-dependent ATPase activity. Confocal microscopy and FRAP analyses revealed that Ser655Arg displayed slow fluorescence recovery and markedly reduced mobile fractions at the lamellipodia. Notably, unlike other variants, Ser655Arg did not form hetero-filaments with NMIIA or NMIIB. All the motor domain mutants disrupted lamellipodial localization and induced blebbing phenotype in human MDA-MB-231 breast cancer cells, but such effect was statistically more significant with Ser655Arg. Altogether, these data suggest that the Ser655Arg mutation at loop 2 of NMIIC motor domain displays biochemical properties that enable the cells to maintain plasticity and facilitate cancer progression.