Structural anomalies in the Eph receptor due to clinically relevant mutations and the subsequent effect on kinase domain

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Eph (erythropoietin producing hepatocellular) receptor constitutes the largest family of receptor tyrosine kinase. Based on sequence homology and binding partners, Eph receptor and Ephrin ligand are classified into EphA/EphrinA and EphB/EphrinB complexes [1]. Eph-ephrin as a family is ubiquitously expressed in almost all tissue [2]. Both are membrane-bound structures and regulates key physiological events such as cell-cell interaction, cell migration, partitioning and cell adhesion [3]. Eph receptors constitute an extracellular ligand binding domain, a cysteine-rich sushi domain and fibronectin repeat domains. Followed by a transmembrane domain lies the intracellular region of the receptor - juxtamembrane domain, kinase domain (KD) and SAM or PDZ binding domain [4]. Mutations reported in Kinase domain (KD) can affect the overall functionality of the receptor and downstream signalling pathways. Among the different Eph receptors, EphA7 has been recently regarded as a cancer driver gene (cancer gene census, COSMIC database). Similar to other Eph receptors, EphA7 also hold a dual functionality were it can act both as an oncogene and as a tumor suppressor [5, 6]. This dual functionality relate to its varied expression in different cancers. Many clinically important mutations have been reported in EphA7 (cbioportal, cosmic database), among which KD specifically holds hot spot mutations. In the present study, EphA7 mutations, Gly656Arg, Gly656Glu and Asp751His, were selected on the basis of in-silico analysis presented in the cbioportal. Gly656Glu and Gly656Arg are the hotspot mutations and present in the loop connecting two conserved beta sheets at the N – lobe of kinase. The third mutant Asp751His is present on the helix of C – lobe near to the catalytic loop. G656R, G656E, D751H have been crystallized and the structure is solved at a resolution of 3.1Å, 2.6Å, 3.05Å with the Rfactor/Rfree – 0.244/.280, 0.181/0.21, 0.199/0.247 respectively. Significant alterations in kinase domain has been observed due to the mutations that can affect binding affinity of ATP as well as catalytic efficiency of the Kinase Domain. Changes at the secondary structure levels were also observed in the hinge region for Gly656Arg and Asp751His mutants. This can adversely affect the transition of Kinase Domain from open to closed or closed to open confirmations.

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Keywords: RTKs; crystal structure; secondary structural changes; mutations; cancer; change in intramolecular interaction

Acknowledgement – Funding for this study was supported by Annual Scientific Fund from ACTREC-TMC. S.C. is thankful to Council for scientific and industrial research (CSIR) for fellowship. The authors thank the XRD facility at ACTREC for providing necessary support to this study.