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CASE REPORT

A Novel 28bp Deletion in Vegfr-1/Flt1 in a 60 yr old patient diagnosed with CRC: a case report

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ABSTRACT

Receptor tyrosine kinases (RTKs) are considered as most effective targets for development of anti-cancer therapies. However, most of the RTKs are mutationally altered in cancers which could either develop resistance or differential sensitivities to RTK-targeted therapies signifying role of mutational profiling in cancers. A 60-year old man was presented with 15days persistency of abdominal pain, disturbed bowel habits and rectal bleeding. USG of the abdomen revealed mass (5cmx6cm) in the right hypochondrium (RHC) seemingly arising from colon. Contrast Enhanced Computed Tomography (CECT) confirmed presence of a polyploid mass (4cmx4cm) in the region of hepatic flexure of colon with adherence to liver. Pathological observation of the colonic biopsy diagnosed the patient with stage II CRC and referred for surgical resection of the colonic lesion. Genotyping of the biopsy tissue revealed a major (28bp) deletion in the putative N-terminal tyrosine kinase (TK) domain of VEGFR-1 gene. In silico analysis revealed major structural and energy changes in the mutant protein. This is the first report to document such a major 28bp deletion in TK domain of VEGFR-1/Flt1 in colorectal tumors, the mutants of which are otherwise considered as embryonically lethal. Since, the deletion occurs in the protein kinase domain and extends over ATP-binding domain and important tyrosine residues of the receptor; it may have possible functional implications in tumor survival.

Keywords: Colorectal cancer, VEGFR-1, Tyrosine Kinase domain

Abbreviations: RTK – Receptor Tyrosine Kinase; CRC – Colorectal cancer; Flt1 Fetal Liver Kinase-1; VEGFR – Vascular Endothelial Growth Factor Receptor.

1. INTRODUCTION

Receptor Tyrosine Kinases (RTKs) are the major class of cell surface regulating diverse signaling pathways in cell growth, differentiation and survival. Mutational analysis of these kinase genes provide attractive targets for therapeutic intervention which have been successfully demonstrated for BRC-ABL tyrosine kinase in leukemic patients (An et al., 2010) or formulation of personalized therapeutics based on the kinases that are mutationally altered in individual cancers e.g Ras mutations (Ross et al., 2015). VEGFR 1 is a member of RTKs and an important regulator of endothelial cell proliferation particularly in tumor angiogenesis (Schwarts et al., 2010). The kinase domain of VEGFR-1 is of prime importance owing to its catalytic nature and is considered as a major determinant of cellular signaling mediated by the receptor. Gene mutations have been predominantly reported in CRC (Yu et al., 2010) and most of them are believed to be activating in nature based upon their position in the key domains (Kloth et al., 2015). Here, we report a novel 28bp deletion in TK domain of VEGFR-1/Flt1 gene in a 60yr old CRC patient.

2. CASE REPORT

A 60yr old male complaining of abdominal pain, disturbed bowel and rectal bleeding was presented to the oncology unit. The patient placed under observation showed persistence of chief complaints for 15 days post hospitalization. USG of the abdomen revealed mass (5cmx6cm) in the right hypochondrium (RHC) seemingly arising from colon. The patient was thoroughly screened by Digital rectal examination (DRE), Proctosigmoidoscopy, Colonoscopy and Contrast Enhanced Computed Tomography (CECT) which confirmed presence of a polyploid mass (4cmx4cm) in the region of hepatic flexure of colon with adherence to liver. Serological analyses showed blood CEA levels of 47ng/ml, Hb 9g/dl with normal WBC count. Pathological examination of the biopsy from the colonic lesion categorized the tissue as stage II well differentiated adenocarcinoma. Genotyping of the colonic lesion and its corresponding control tissue was performed for TK domain of VEGFR-1 using gene specific primers. Successfully genotyped target gene loci upon sequencing (Sanger double-pass sequencing) revealed a major 28bp deletion corresponding to the gene loci NC_000013.10:g.28913327_28913354 on Chr 13 (KF002713), figure 1.

3. *IN SILICO* ANALYSES

3D models of Mu VEGFR-1 were built using i-Tasser and SwissProt protein modeling servers separately against protein template 3HNGA (serving as WT VEGFR-1) corresponding to putative tyrosine kinase domain of VEGFR-1. Average prediction models from the two servers were used to study effect of deletion on protein structure, energytransformation and molecular changes.The Accelrys Discovery Studio (Accelrys v4.1 2014) v 4.1 used to visualize molecular and energy changes in Mu compared to WT VEGFR-1 model (3HNGA). As depicted in Figure 2A, a substantial loss of secondary structure was observed in the C-terminus region corresponding to β-sheets; however, the overall structure of the Mu remained stable with minor changes in torsional angles. A decrease in net formal charge (Wt: 2; Mu: -3) was also observed. Figure 2B shows a major reshuffle in the interpolated charges surface of amino acids in Wt and Mu VEGFR-1.

4. DISCUSSION

Rare mutations (present in only a small percentage) are not only important but may contribute to natural selection process during the course of tumorigenesis as well as determine treatment outcome in certain cases (Leroi et al., 2003). Thus, a prior knowledge of the presence of a clinically or biologically relevant mutation can provide logical insights into disease progression and could help designing rational interven -tion to circumvent tumor growth. Mutational profiling of cancers provide a molecular snapshot of mutations in each individual tumor type type for understanding the signaling pathways involved in driving cancer growth and, eventually, to devise the best therapeutic strategy for each individual specifically, commonly called the “personalized medicine”. VEGFRs are considered key determinants of tumor angiogenesis, particularly VEGFR-1 which has been reported to induce metastasis and has been associated with shorter survival in many cancers (Ross et al., 2015). TK domain of VEGFR-1 harbors prime tyrosine residues necessary for VEGFR-1 signaling and functions to transmit signaling induced by VEGF – A and –B, leading to activation of key processes regulating cell migration, metastasis (Duffy et al., 2004) and thus could be decisive in cancer progression. In this study, we report a rare 28mer deletion in the putative tyrosine kinase (TK) domain of VEGF receptor-1 in a 60year old patient diagnosed with cancer of the colon. As the patient didn’t had any previous familial history of cancer nor received any chemo- or radiotherapy, it is plausible that the deletion has occurred de novo. Intriguingly, the 28bp deletion results in generation of a TK truncated VEGFR-1, although forming a stable protein as revealed in silico, such VEGFRs had been established as embryonically lethal. This is the first clinical case to report such a major deletion in colon cancer. Since, the deletion occurs in the protein kinase domain and extends over ATP-binding domain and important tyrosine residues of the receptor; it may have possible functional implications in tumor survival.

5. ETHICS STATEMENT

This study has been approved by the Institutional Ethical Committee, SKIMS Srinagar, IND. The study was conducted on the colonic samples after obtaining the written informed consents from the patients.

SUMMARY OF RESEARCH

1. Colon cancers are considered as having most frequently mutated phenotype as reported by a large body of literature and in human genomic variation databases like dbSNP, ClinVar etc.

Taseem A. Mokhdomi et al.
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2. These mutations could be inherited upon generations or could arise *de novo* during carcinogenesis predisposing cells either towards cancer activation or inhibition depending upon their nature thus signifying mutational screening in cancers.
3. We present a novel case of an elderly colon cancer patient with a rare 28bp deletion in the kinase domain of VEGF receptor 1. *In silico* analysis confirmed stable nature of the mutation with minor changes in torsional angles and net formal charge of the predicted protein.
4. Although VEGFR-1 is presumably decoy in characteristics, kinase deletion mutants of VEGFR-1 are considered lethal, thus our findings cogitate an alternative mechanism of VEGFR-1 signaling.

FUTURE ISSUES

Our findings present a rare case of stable kinase mutation in colorectal cancer. It would rather be more interesting to study such a mutant phenotype in appropriate *in vitro* models, particularly in processes like angiogenesis, where VEGFR-1 plays an active part.

DISCLOSURE STATEMENT

There is no special financial support for this research work from any funding agency. All the authors declare no conflict of interests.

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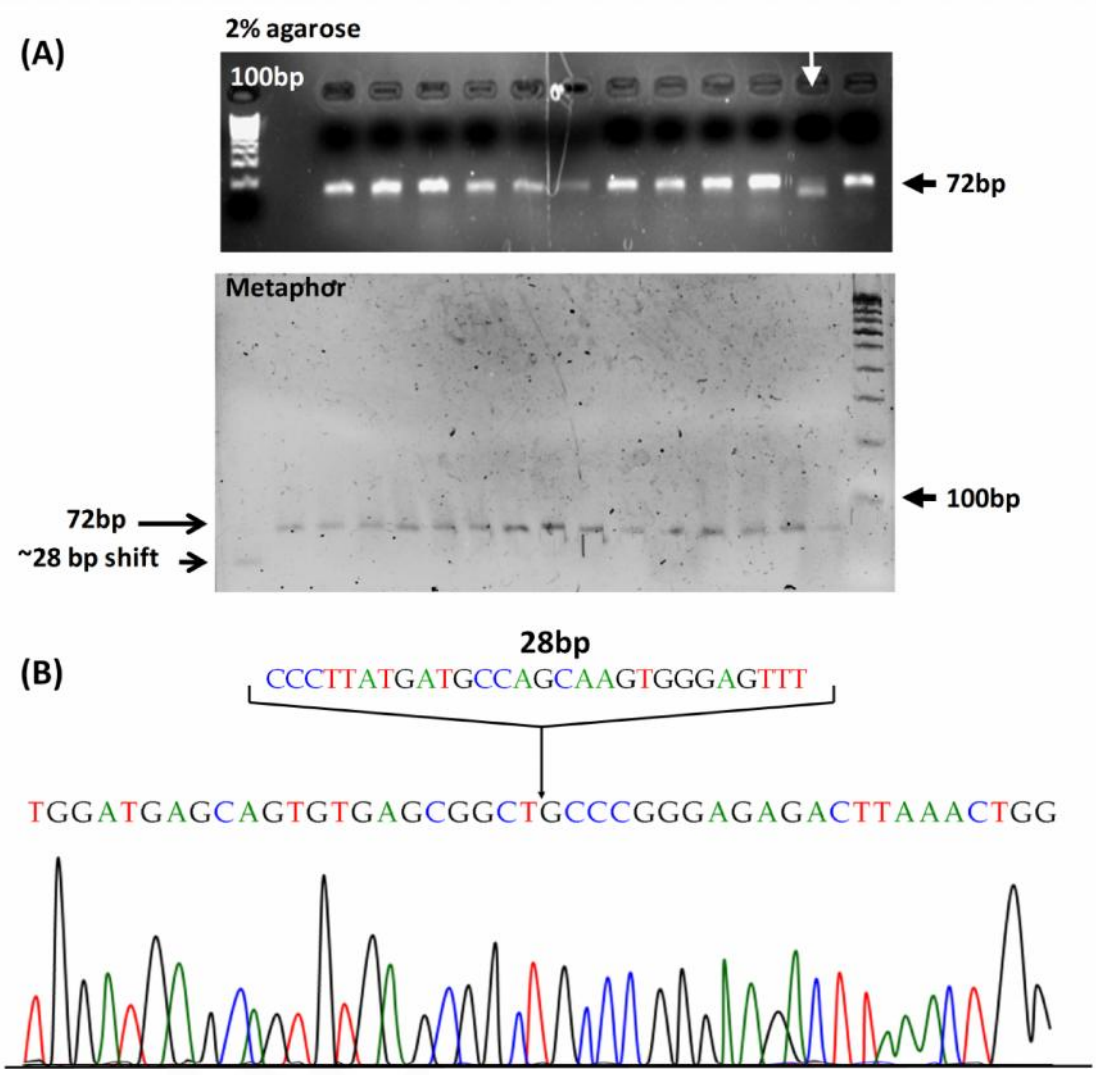

Figure 1
28bp deletion in FLT-1 gene A) Agarose gel and Metaphor analysis B) Sequencing chromatogram

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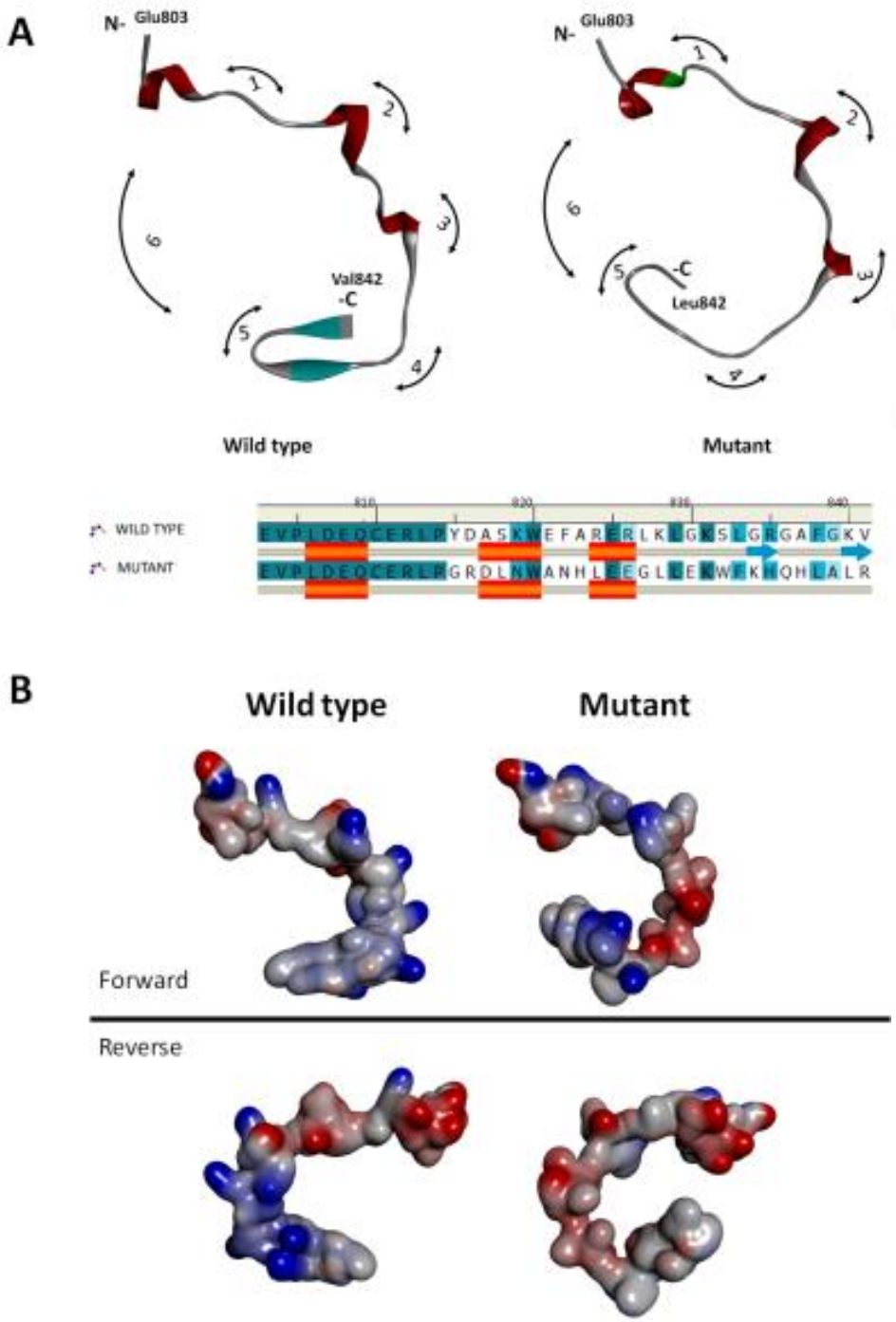

Figure 2

Comparison of Wt VEGFR-1 and predicted 3D model of Mu VEGFR-1 generated using i-Tasser and SwissProt protein modeling servers showing amino acids position 803 – 842 corresponding to putative TK domain. (A) Arrows Indicate torsional changes (B) Interpolated charge surface of Wt and Mu VEGFR-1 (Red: acidic; Blue: Basic; Grey: Neutral) shown in forward orientation and reverse orientation.

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