

Population-specific genetic variation at microRNA-629-binding site in the 3'-untranslated region of NBS1 gene in Ovarian Cancer Patients

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ABSTRACT

Objective: MicroRNAs (miRNAs) have emerged as key gene regulators of wide ranging biological pathways and early studies have shown that miRNA expression is misrepresented in cancer and experimental data substantiates the fact that cancer phenotypes can be modified by targeting miRNA expression. Data obtained through high-throughput technologies is deepening our understanding about the fact that miRNAs bind to target sequences in mRNAs, typically resulting in repressed gene expression. It has been convincingly revealed that loss or gain of miRNA function can be caused by a single point mutation in either the miRNA or its target or by epigenetic silencing of primary miRNA transcription units.

Methodology: 3'-UTR of NBS1 gene was genotyped in a Pakistani Ovarian cancer case-control population including 20 cases and 10 controls using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis.

Results: In the present laboratory research, we studied 3' UTR C/T polymorphism in NBS gene in 20 ovarian cancer patients and 10 controls. The results indicated that out of 20 patients 8(40%) were of TT genotype. 6(30%) were homozygous for C and 6(30%) were of CT genotype. Genotyping of age and sex matched controls revealed that out of 10, 3(30%) were of CT genotype. 4(40%) were CC and 3(30%) were TT.

Conclusion: NBS is an important component of DNA damage repair signaling networks, and a better knowledge of how mutations influence protein networks is an important step in understanding cancer progression.

Keywords: Ovarian cancer, NBS1 gene, microRNA-629

INTRODUCTION

Reconceptualization of phenotype and genotype-driven studies provide considerable evidence that miRNAs control carcinogenesis through divergent or convergent regulation of oncogenic pathways. Rapidly accumulating evidence suggests that some miRNA regulatory networks govern cell-autonomous cancer phenotypes and importantly, polymorphisms at the microRNA-binding sites may influence the binding ability of microRNA and its posttranscription modulation on gene expression and thus contribute to disease susceptibility. miRNAs are further characterized into subcategories of tumor suppressor and oncomirs. Cell type specific misrepresented miRNA subsets are studied and reviewed in details Farooqi et al, 2013, Fayyaz et al, 2013, Farooqi et

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al,2012. Ovarian cancer associated miRNA dysregulation is also extensively reviewed Kuhlmann et al, 2012; Mezzanzanica et al, 2011. In response to DNA damage, cells activate a complex, kinase-based signaling network to arrest the cell cycle and allow time for DNA repair that includes transient recruitment of the MRE11/RAD50/NBS1 (MRN) complex at DSB sites, followed by the recruitment/activation of ataxia–telangiectasia mutated (ATM) kinase. Research over the years has provided a broader landscape of DNA damage repair signaling network, that primarily consists of two components—a rapid phosphorylation-driven signaling cascade that results in immediate inhibition of Cdk/cyclin complexes and a delayed transcriptional response. In this preliminary research work we studied germ line mutation in the 3'-UTR of NBS1 in ovarian cancer patients.

MATERIALS AND METHODS

A C/T polymorphism in the 3'-UTR of NBS1 gene was analyzed for any association between the genetic variations and ovarian cancer risk. Pakistani Ovarian

cancer case-control population including 20 cases and 10 controls using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis. Briefly, primers (forward: 5'-ATTGATATGTATGTTGTGAAGTA-3' and reverse: 5'-AAAGTCCAGAAACAGATCCACCA-3') and PCR product was digested by RsaI to identify the rs14448T>C.

RESULTS

| NBS1 Genotypes | CT | CC | TT |
|----------------|-----|-----|-----|
| | 6 | 6 | 8 |
| | 30% | 30% | 40% |

RFLP results of NBS gene in ovarian cancer patients

| NBS1 Genotypes | CT | CC | TT |
|----------------|-----|-----|-----|
| | 3 | 4 | 3 |
| | 30% | 40% | 30% |

RFLP results of NBS gene in controls: In the present laboratory research, we studied 3' UTR C/T polymorphism in NBS gene in 20 ovarian cancer patients and 10 controls. The results indicated that out of 20 patients 8(40%) were of TT genotype. 6 (30%) were homozygous for C and 6(30%) were of CT genotype. Genotyping of age and sex matched controls revealed that out of 10, 3(30%) were of CT genotype. 4(40%) were CC and 3(30%) were TT.

DISCUSSION

miRNA mediated control of mRNA transcripts has emerged as an exciting avenue of research and tremendous experimental evidence is substantiating positive and negative relationships of miRNA circuitries in regulation of cellular activities. More importantly, miRNA regulated control of DNA damage regulators is also developing our comprehensions about the fact that DNA damage repair is also under direct control of miRNA subsets. miR-182-5p is overexpressed in cancer cells and has been shown to negatively regulate BRCA1 Krishnan et al, 2013. Similarly, ATM is post-transcriptionally controlled by miR-421 Hu et al, 2010. Although a previous report reveals lack of association between rs14448 and lung cancer in Southern and Eastern Chinese population however those findings cannot be extrapolated to

other populations due to intra- and inter-ethnic variability. TT genotype was 58% in cancer cases and 55.7% in controls. TC genotype was 35.9% in cancer patients and 36.8% in controls. CC genotype was 6.1% in cancer patients and 7.5% in controls.

We do not have better information about the expression of NBS1 and miRNA subsets in ovarian cancer patients of our local population. In future we plan to investigate expression profile of miRNA subsets so that we can develop a relation between miRNA control of NBS1 and ATM.

REFERENCES

1. Fayyaz S, Farooqi AA. miRNA and TMPRSS2-ERG do not mind their own business in prostate cancer cells. *Immunogenetics*. 2013 May;65(5):315-32.
2. Farooqi AA, Nawaz A, Javed Z, Bhatti S, Ismail M. While at Rome miRNA and TRAIL do whatever BCR-ABL commands to do. *Arch Immunol Ther Exp (Warsz)*. 2013 Feb;61(1):59-74.
3. Farooqi AA, Naqi A, Qureshi MZ, Rana A, Khan A, Riaz AM, Afzal SM, Rasheed N, Bhatti S. Prostate cancer is known by the companionship with ATM and miRNA it keeps: craftsmen of translation have dual behaviour with tailors of life thread. *Cell Biochem Funct*. 2012 Oct;30(7):611-7.
4. Kuhlmann JD, Rasch J, Wimberger P, Kasimir-Bauer S. microRNA and the pathogenesis of ovarian cancer--a new horizon for molecular diagnostics and treatment? *Clin Chem Lab Med*. 2012 Jan 13;50(4):601-15.
5. Mezzanzanica D, Canevari S, Cecco LD, Bagnoli M. miRNA control of apoptotic programs: focus on ovarian cancer. *Expert Rev Mol Diagn*. 2011 Apr;11(3):277-86.
6. Yang L, Li Y, Cheng M, Huang D, Zheng J, Liu B, Ling X, Li Q, Zhang X, Ji W, Zhou Y, Lu J. A functional polymorphism at microRNA-629-binding site in the 3'-untranslated region of NBS1 gene confers an increased risk of lung cancer in Southern and Eastern Chinese population. *Carcinogenesis*. 2012 Feb;33(2):338-47.
7. Krishnan K, Steptoe AL, Martin HC, Wani S, Nones K, Waddell N, Mariasegaram M, Simpson PT, Lakhani SR, Gabrielli B, Vlassov A, Cloonan N, Grimmond SM. MicroRNA-182-5p targets a network of genes involved in DNA repair. *RNA*. 2013 Feb;19(2):230-42.
8. Hu H, Du L, Nagabayashi G, Seeger RC, Gatti RA. ATM is down-regulated by N-Myc-regulated microRNA-421. *Proc Natl Acad Sci U S A*. 2010 Jan 26;107(4):1506-11.