Whole Genome Comparative Genomic Hybridization of Ewing Sarcoma Indicates Cytoskeleton, Migration and Protein Trafficking [†]

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Abstract: Ewing sarcoma is a bone and soft tissue tumor either neuroectodermal or mesenchymal originated and affecting children and adolescents. In the present study, we aimed to find out prognostic and predictive biomarkers for Ewing sarcoma. Hence, we examined the copy number alterations (and related possible genes) among ten Ewing sarcoma patient samples and possible associations with the clinical outcome. DNA extraction from formalin fixed paraffin embedded archive tissues was performed. Whole genome Comparative Genomic Hybridization (CGH) was performed by NimbleGen and recorded as single Panel Rainbow through chromosomes 1–22, X and Y. Data was interpreted by SignalMap software and genetic regions matching the deletion or amplification loci were recorded. The mean age of the patients was 8.6 years. Three of the cases were male, while seven were female. According to CGH analysis, the most common DNA copy number alterations were found in SLIT-ROBO Rho GTPase activating protein (srGAP2), RANBP2 like GRIP domain (RGPD5), nephrocystin 1 (NPHP1), GTF2I repeat domain containing 2 (GTF2IRD2), pyridoxal dependent decarboxylase domain containing 1 (PXDC1), which were found down-regulated among 7 of 10 patients. In conclusion, in our dataset the copy number alterations are mostly found in genes related with cytoskeletal elements, migration and protein trafficking among our patient group. Gene functional studies are required for better understanding the role of these genes in Ewing Sarcoma pathogenesis

Keywords: Ewing sarcoma; CGH array; chromosomal copy number alterations

1. Introduction

Ewing sarcoma is a bone and soft tissue tumor either neuroectodermal or mesenchymal originated and affecting children and adolescents [1]. About 20–25%, cases are metastatic at the diagnosis and long-term survival are poor for metastatic or relapsed patients [1]. The diagnosis of tumor is based on small round blue cell tumor, which is stained by CD99 [2]. In addition, most of the cases have translocation between t (11; 22) (q24; q12) in which amino terminus of EWSR1 gene and carboxyl terminus of FLI1 gene [3]. The fusion gene leads to oncogenic activation, epigenetic remodeling. There are also other type of translocations such as t (21; 22) (q22; q12) EWS-ERF fusion in less amount of cases [3]. In previous studies STAG2, CDKN2A, TP53 mutations were found in Ewing Sarcoma patients [4].

Microarray based comparative genomic hybridization are used for screening submicroscopic DNA changes between tumor and reference DNAs and detecting gains and losses between DNAs [5]. In our study, we have applied CGH technology for detecting copy number variation among Ewing Sarcoma patients and examined the genomic changes.

2. Materials and Methods

Total 10 Ewing sarcoma patient samples were collected between 1998–2011, from archives of Behcet Uz Pediatric Hospital, Izmir Turkey. DNA isolation was made from FFPE tissue samples. Roche Nimble Gen Comparative Genomic Hybridization array was used for detecting copy number alterations. The patients' DNA and normal control DNA (Human Genomic DNA) were labelled with Cy3 and Cy5 each through a random priming method, using Panel Rainbow through chromosomes 1–22, X and Y. (NimbleGen Dual-Color DNA Labeling Kit, Roche NimbleGen, Inc., Madison, WI, USA). Array hybridization and washing steps were executed on NimbleGen 135K CGX-3 Array according to manufacturer's instructions. The results were analyzed with using Signal Map Software (Roche Nimble Inc., Madison, WI, USA). The aberrations in DNA copy number were detected. The study was approved from Dr.Behcet Uz Children's Research Hospital Ethical committee.

3. Results

Properties of Cases: Totally 10 Ewing sarcoma patients were analyzed in which three of them male and seven of them female. The mean age of the patients was 8.6 years. The oldest patient was 14 years old while the youngest was one year old. The tumors were located in different body parts (Table 1).

Table 1. Clinical features of each patient.

Patients	Clinical Features
1A1	4 years, female, spinal area
1A3	11 years, male, right thorax
1A5	1 year, male, pre-sacral
1A7	14 years, female, lower extremity
1A8	13 years, female, spinal area
1A12	7 years, female, thorax wall
2A1	13 years, male, abdominal wall
2A4	4 years, male, shoulder
2A5	8 years, female, left pleura
2A6	11 years, female, abdominal wall

CGH Anaysis Results: According to CGH analysis, the most common DNA copy number alterations were found in SLIT-ROBO Rho GTPase activating protein (srGAP2), RANBP2 like GRIP domain (RGPD5), nephrocystin 1 (NPHP1), GTF2I repeat domain containing 2 (GTF2IRD2), pyridoxial dependent decarboxylase domain containing 1 (PXDC1), which were found down-regulated among 7 of 10 patients. This was followed by MALL, Ankyrin Repeat Domain 30A (ANKRD30A) and protein tyrosine phosphatase non receptor type 20 (PTPN20) and ubiquitin specific peptidase 22 (USP22), transmembrane phosphatase with tensin homology (TPTE), which were found in 6 of 10 patients (Table 2).

Table 2. The most common DNA copy number aberration and corresponding gene.

Symbol	Gene Name	Location	1A1	1A3	1A5	1A7	1A8	1A12	2A1	2A4	2A5	2A6	Total
srGAP2	SLIT-ROBO Rho GTPase-activating protein 2	1q32.1	del	del	del	del	del	x	del	del	x	x	7/10
RGPD5	RANBP2-like and GRIP domain containing 5	2q13	del	del	x		del	del	del	del	del	x	7/10
NPHP1	nephrocystin 1	2q13	del	x	x	del	del	del	del	del	del	x	7/10
GTF2IRD2	GTF2I repeat domain containing 2	7q11.23	del	del	x	del	x	del	del	del	amp	x	7/10
PDXDC1	pyridoxal dependent decarboxylase domain containing 1	16p13.11	del	del	x	del	del	del	x	del	del	x	7/10
MALL	T cell differentiation protein like	2q13	del	del	del	del	del	x	del	x	x	x	6/10
ANKRD30A	Ankyrin repeat domain 30A	10p11.21	del	del	x	del	x	del	x	del	del	x	6/10
PTPN20	PTPN20 protein tyrosine phosphatase, non-receptor type 20	10q11.21	del	del	del	x	del	x	x	del	del	x	6/10
USP22	ubiquitin specific peptidase 22	17p11.2	x	del	x	del	x	del	del	del	del	x	6/10

4. Discussion

CGH array is a useful method for detection of DNA copy number alterations among Ewing sarcoma patients. In our patient cohort, srGAP2, RGPD5, NPHP1, GTF2IRD2, PDXDC1 are found as most copy number altered genes. srGAP2 is a member of SLIT-ROBO Rho GTPase activating protein family, which regulates actin dynamics in cell migration, cell differentiation and neuronal migration. The gene is also located in the region 1q. The deletion in 1q regions were observed in previous CGH analysis as well [6]. RGPD5 is a nuclear membrane associated protein and regulates cellular functions through interactions with other proteins. NPHP1 is interacting with Crk associated substrate and functioning in controlling cell division, cell-cell and cell-matrix adhesion signaling. GTF2IRD2 is a transcriptional factor under the control of Retinoblastoma protein. MALL is participating in raft mediated trafficking in endothelial cells. MAL1 interacts with caveolin and localizes in glycolipid or cholesterol enriched membrane rafts. PTPN20 is a member of protein tyrosine phosphatases and involving in actin polymerization. USP22 is an interesting gene and regulates various cellular and organismal processes such as cell proliferation, apoptosis and malignancy. It also deubiquitinates histones H2A and H2B, thereby acting as coactivator. USP22 participates in activation of several oncogenes such as MYC and contributing to stemness in colorectal carcinogenesis as well as epithelial mesenchymal transition in osteosarcoma cell lines. ANKRD30A expressed in epithelial cells and in testis, its altered expression levels is found to be associated with breast cancer. TPTE is PTEN related tyrosine phosphatase and plays role in endocrine signal transduction pathways and in spermatogenic function of testis. CTSE encodes peptidase that involves in antigen processing and maturation of secretory proteins [7,8].

5. Conclusions

In conclusion, in our dataset the copy number alterations are mostly found in genes related with cytoskeletal elements, migration and protein trafficking among our patient group. Gene functional studies are required for better understanding the role of these genes in Ewing Sarcoma pathogenesis.

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