Study of comparative proteome between normal and inverted karyotypes of human mesenchymal stem cells

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ABSTRACT. Multipotent mesenchymal stem cells have been expanded in vitro for cellular therapy in numerous clinical settings without standardized culture conditions or quality-control schemes. The in vitro expansion is necessary to obtain sufficient cells for clinical applications. However, the expansion may induce genetic and functional abnormalities which may affect the safety and functionality of MSC, especially the chromosomal stability. This study aimed to investigate the protein profile of umbilical cord-derived MSC with normal and inverted karyotypes after expansion in the laboratory. Mass spectrometry analysis was performed and the Bradford method, Scaffold software, String and Cytoscape databases were employed to measure and characterize the protein content of umbilical cord-derived MSC. Networks of protein interactions, hub and bottleneck proteins were identified by proteomics and systems biology approaches. We found that proteins related to cellular stress were super expressed in inverted karyotype cells. Moreover, a high expression of Serpine 1, RHOA, and CTSB was found in these cells, which are proteins related to cancer. The albumin and ubiquitin proteins have been associated with a positive prognosis in cancer and cellular stress, and were up- and down-regulated in normal karyotype cells, respectively. The results suggests that the paracentric inversion inv(3)(p25p13) induced some type of cellular stress and genetic instability in human mesenchymal stem cells. These analyses showed the importance of carrying out studies related to the genetic instability of human mesenchymal stem cells using the protein expression profile as a parameter.

Keywords: genetic instability; protein profile; umbilical cord; interaction network.

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Introduction

Human umbilical cords (UCh) have been considered a biological risk residue after birth. However, UCh have been an interesting source of mesenchymal stem cells (MSC) for being disposable and accessible (Yun et al., 2016). Mesenchymal stem cells from the human umbilical cord (MSC-UCh) can be used without raising any ethical issue since these cells are a generally discarded extra-embryonic tissue. MSC-UCh have a multipotent differentiation, proliferate fast and have a close ontogenetic relationship with embryonic stem cells (He et al., 2016). Therefore, the UCh is an excellent source of MSC and can be used in regenerative medicine, for example.

Human MSC have been the subject of studies in the field of regenerative medicine and bioengineering because of their capacity for self-renewal and differentiation in other cell types. However, these cells need to be expanded in order to obtain a suitable number of cells used in treating diseases (Fan, Zhang, & Zhou, 2011). MSC expansion requires time, and during this time chromosomal alterations such as chromosomal inversions and modification of cell characteristics can occur. Chromosomal inversions may lead to genetic instability and increase the risk of producing abnormal gametes. Abnormal gametes can lead to unbalanced offspring, with duplications and deficiency of inverted chromosome segments (Vieira & Ferrari, 2013).

Paracentric inversions are balanced chromosomal rearrangements involving two breaks in the same chromosomal arm, followed by a 180° rotation of the chromosomal segment and its re-insertion. Paracentric inversions do not include the centromere. Most inversions have unique breakpoints and the inversion incidence in humans is rare, being estimated at 0.1-0.5 1,000⁻¹ in the population. Paracentric inversions have been described in all human chromosomes, but they are most common in chromosomes 1, 3, 5, 6, 7, 11, and 14 (Rigola et al., 2015).
Balanced paracentric inversions generally appear to be harmless. Balanced inversions are usually clinically asymptomatic because they do not involve a quantitative variation of the genetic material. However, infertility, spontaneous abortions and cognitive deficit have been reported in some patients with this type of inversion. On the other hand, cytogenetic molecular methods are necessary to detect or to rule out the presence of unbalanced chromosomal alterations, especially when dealing with MSC used in gene therapies (Rigola et al., 2015).

In a previous study, our group found a paracentric inversion in the short arm of chromosome 3 (3p25-26) in MSC isolated from one umbilical cord. In this context, studying the MSC-UCh proteins is relevant since these molecules are directly or indirectly responsible for controlling all or almost all biological processes (Barbosa et al., 2012). Proteomics studies the set of proteins in a descriptive and quantitative way, as well as how the protein levels vary in the population depending on the environment or interactions with other proteins (Valledor & Jorrín, 2011). The proteome is dynamic and changes according to the physiological status and cell differentiation phases (Barbosa et al., 2012). In a quantitative proteomic analysis, 463 surface proteins were found in MSC submitted to differentiation in osteoblasts (Foster et al., 2005). Furthermore, 1,001 surface proteins were found in another quantitative analysis using MSC from bone marrow (Lee et al., 2013). Finally, an experiment found 1664 proteins in MSC, as 607 proteins were obtained from bone marrow and 1052 proteins were obtained from the nerve tissue (Bryukhovetskiy et al., 2014).

Systems biology is a tool used to build protein-protein interaction networks in order to understand the interactions between these molecules. Systems biology enables constructing mathematical models, simulations, data processing techniques, and integrating information, thereby achieving a better understanding of the interactions between the living systems components and their biological processes (Mesquita, Jorge, Souza Junior, & Cassino, 2014). Therefore, systems biology was used in order to analyze the paracentric inversion in MSC-UCh at a protein level. Overall, this research aimed to analyze the protein expression profile of human MSC with normal and inverted (inv(3)(p25p13) karyotypes in order to characterize and compare these cells.

**Material and methods**

**Isolation of MSC-UCh**

This work was submitted and approved by the Ethics Committee of the Universidade Federal do Rio Grande do Norte (CEP/UFRN No. 044.0.051.000-07). Umbilical cord specimens were aseptically obtained by doctors after written informed consent was signed by mothers. The umbilical cord was maintained in PBS buffer in the Laboratory of Molecular and Genomic Biology of the Universidade Federal do Rio Grande do Norte (LBMG/UFRN). The UCh was washed with PBS buffer to remove excess blood and other contaminants. After this washing, the UCh was cannulated and a 0.5% solution of type IV collagenase was introduced for endothelium enzymatic breakdown (40 minutes, 37°C). After disaggregation, the reaction was inhibited by adding fetal bovine serum (FBS) into the UCh vein. The cell wall suspension was collected on a Petri dish, placed in tubes and centrifuged at 2,000 rpm for 10 min. The supernatant was discarded and the cells’ pellet was suspended with DMEM-low glucose supplemented culture medium (20% FBS and 1% antibiotic). The cell solution was transferred to culture flasks, which were kept in an incubator (37 °C, 5% CO₂) for 48 hours in order for the cells to adhere to the vials.

**MSC characterization**

The cell solution was sent to the Laboratory of Immunogenetics - Department of Biochemistry in the Universidade Federal do Rio Grande do Norte for flow cytometry by a FACSCanto II, BD cytometer. We performed flow cytometry and osteogenic, chondrogenic and adipogenic differentiation in order to characterize the cells as MSC. MSC are generally positive for CD105, CD90, and CD73 surface markers and negative for HLA-DR, CD45, CD34, and CD14 surface markers. In addition, MSC are able to differentiate into osteoblasts, chondrocytes, and adipocytes. The differentiation can be observed by staining the cells. The osteoblasts are stained with Alizarin red; chondrocytes are stained with Alcian blue and adipocytes are stained with Oil red (Dominici et al., 2006). A beta-galactosidase test was performed in order to verify if the cells used were in the senescence process. It is possible to see if the cells express beta-galactosidase through cell staining (blue cells are senescent) (Shevchenko, Wilm, Vorm, & Mann, 1996).
Cell culture

We used six samples. A cell culture was performed in two MSC lines. These cells were cultured in alpha-MEM culture medium supplemented with 10% FBS, 1% antibiotic, and 1% glutamine until the culture was approximately 90% confluent. The culture was washed with PBS. The cell pellet was dissolved in a lysis buffer containing 7M urea, 2M thiourea, 4% CHAPS, 50mM Tris–HCl (pH 8.5), and 50mM DTT. The cell extract was resuspended, centrifuged for 20 minutes (4 °C at 12,000 RPM), and the supernatant (protein extract) was collected. The proteins were measured by the Bradford method.

Mass spectrometry and protein expression

The proteins were digested enzymatically (In vitro digestion protocol: Anal. Chem., 1996 68: 850-858) and separated for mass spectrometry analysis. The protein extract was sent to the Mass Spectrometry Laboratory of the National Laboratory of Biosciences (CNPEM-ABTLuS) in the Universidade de Campinas (UNICAMP) for mass spectrometry analysis by Q-Tof spectrometer.

Scaffold software (http://www.proteom software.com/products/scaffold/) was used to quantify and classify the proteins according to their profile (Scaffold Elements, version 2.1.1). The raw data from mass spectrometry were processed using Scaffold software, and the Fold Change value was obtained. The Fold Change value was used to classify the proteins as up-regulated and down regulated. In this context, we evaluated the proteins up-regulated and down-regulated expressed in cells with a normal karyotype, in cells with an inverted karyotype, and in both cell types.

Protein functions

The protein identification numbers (ID) generated by Scaffold software were converted into identification numbers compatible with the String database through the UniProt ID conversion tool (http://www.uniprot.org/uploadlists/). We then obtained six protein lists using Scaffold, including up-regulated proteins and down-regulated proteins of normal karyotype cells, and up-regulated proteins and down-regulated proteins of inverted karyotype cells. The lists were imported into the String database, which provided the molecular functions (MFs) and biological processes (BPs) of proteins (http://string-db.org/cgi/input.pl).

We analyzed the molecular functions (MFs) and biological processes (BPs) of down-regulated and up-regulated proteins of normal karyotype cells. These same steps were repeated for inverted karyotype cells.

Results

No surface markers have been exclusively associated with MSC to date. Based on a proposal by the International Society for Cell Therapy, cells may be classified as MSC if they adhere to plastic, carry a minimal subset of characteristic surface markers (CD73, CD90, CD105) and present the potential to differentiate into bone, fat, and cartilage (Dominici et al., 2006). The cells isolated from the UCh vein were positive for CD105, CD90, and CD73 surface markers, and negative for HLA-DR, CD45, CD34, and CD14 surface markers. The cells were also able to differentiate into the three well-defined cell types: osteoblasts, chondrocytes, and adipocytes (Figure 1). Therefore, the analyzed cells are MSC.

Analysis of protein functions

Q-Tof spectrometry identified 321 expressed proteins, 15 were only expressed in Inverted karyotype cells (Table S1), 42 were only expressed in Normal karyotype cells (Table S2) and 264 were expressed in both cell types (Table S3). In addition, regarding the 264 intersection proteins, 156 proteins were sub expressed and 91 proteins were super expressed in inverted karyotype cells compared to normal karyotype cells.

We analyzed 12 BP and 18 MF overall, where 8 BP were present in normal karyotype cells and 4 BP were present in inverted karyotype cells. Furthermore, 15 MF were present in normal karyotype cells and 3 MF were present in inverted karyotype cells (Figure 2). When comparing inverted karyotype cells and normal karyotype cells, two main BPs were observed: cytoskeleton organization in inverted karyotype cells and
intracellular transport in normal karyotype cells (Figure 2a). We also found cytoskeleton constitution in inverted karyotype cells and RNA binding in normal karyotype cells when analyzing MFs (Figure 2b).

Figure 1. Types of cell differentiation. A) osteogenic: each gap corresponds to 1 mature osteocyte, which communicates with other osteocytes through communicating junctions (not visible); B) adipogenic: orange dots represent adipocytes, their color is derived from a large number of mitochondria and cytochrome oxidase available in the cytoplasm. Adipocyte size varies according to the number of lipid droplets present in the cytoplasm. C) chondrogenic: mature ovoid/rounded chondrocytes with basophilic cytoplasm and few organelles (not visible) in cartilaginous tissue gaps. D) cells without differentiation. Scale: A: 80 µm; B: 5 µm; C: 50 µm; D: 40 µm

Figure 2. Distribution of the main biological processes (A) and molecular functions (B) in normal and inverted karyotype cells. IK, inverted karyotype; NK, normal karyotype.
Some general processes were observed when comparing the up-regulated proteins of normal karyotype cells and inverted karyotype cells and taking into consideration the BPs. We found proteins related to the negative regulation of BP, negative regulation of cellular processes, regulation of BP quality, regulation of apoptotic processes, response to stress, and response to the stimulus. These processes were only found in the inverted karyotype cells. In addition, the translation inhibition, regulation of cell death, tissue regeneration, response to tissue injury, and regulation of body fluids were processes which were only found in normal karyotype cells (Figure 3a).

General BP were also found when analyzing down-regulated proteins. Response to tissue injury, tissue regeneration, and binding proteins were BPs which were only found in inverted karyotype cells. Response to the stimulus, negative regulation of BP, regulation of body fluids, regulation of the immune system, and regulation of BP quality were only found in normal karyotype cells. Down-regulated proteins were related to 10 BPs, but only 2 BPs were common to inverted and normal karyotype cells: the negative regulation of cellular processes and the regulation of apoptotic processes. There is a greater amount of proteins related to these two processes in the inverted karyotype cells when compared to normal karyotype cells (Figure 3b).

**Figure 3.** Quantification of up-regulated (A) and down-regulated (B) proteins related to each BP in normal and inverted karyotype cells. IK, inverted karyotype; NK, normal karyotype; BP, biological process; CP, cellular process.

Regarding the MFs of up-regulated proteins, the activation of structural molecules and cytoskeleton constitution were processes which were only found in inverted karyotype cells. In addition, a group of
binding enzymes was only found in normal karyotype cells. A greater amount of RNA binding proteins was also observed in normal karyotype cells when compared to the inverted karyotype cells (Figure 4a). Regarding the MFs of down-regulated proteins, the activation of structural molecules was only observed in normal karyotype cells. Additionally, a greater amount of RNA binding proteins was observed in inverted karyotype cells when compared to normal karyotype cells (Figure 4b).

**Figure 4.** Quantification of up-regulated (a) and down-regulated (b) proteins related to each molecular function in normal and inverted karyotype cells. IK, inverted karyotype; NK, normal karyotype.

**Protein interaction networks and measures of centrality**

Regarding the protein interaction network of normal karyotype cells, the KRT16 was the bottleneck protein, meaning the articulation point in the network (Figure 5a). The centrality measurement graph shows that KRT16 is the hub protein with the highest betweenness value (Figure 5b). Regarding the protein interaction network of inverted karyotype cells, the RHOA was the bottleneck protein in the network (Figure 5c). The centrality measurement graph shows that RHOA is the protein with the highest betweenness value (hub) (Figure 5d).

Regarding the up-regulated proteins of normal karyotype cells, the GAPDH protein is the articulation point in the network (bottleneck protein) with the highest betweenness value (hub) in the centrality measurement graph (Figure 6a, 6b). Regarding the up-regulated proteins of inverted karyotype cells, the proteins with the highest betweenness-degree were CCT3 and DECR1 (Figure 6d). This result indicates that CCT3 and DECR1 are the network hubs. CCT3 and DECR1 are also the bottlenecks in the protein network (Figure 6c).
Figure 5. a. Protein interaction network of normal karyotype cells. b. Measurements of centrality of normal karyotype cell proteins. c. Protein interaction network of inverted karyotype cells. d. Measurements of centrality of inverted karyotype cell proteins. Thick bars represent strong interactions. Thin bars represent weak interactions.

Figure 6. a. Interaction network of up-regulated proteins in normal karyotype cells. b. Measurement of centrality of up-regulated proteins in normal karyotype cells. c. Interaction network of up-regulated proteins in inverted karyotype cells. d. Measurement of centrality of up-regulated proteins in inverted karyotype cells. Thick bars represent strong interactions. Thin bars represent weak interactions.
Regarding the down-regulated proteins of normal karyotype cells, the UBC protein is clearly the bottleneck in the network, with the highest betweenness-degree value in the centrality measurement graph (Figure 7a, 7b). This result indicates that UBC is the hub. Finally, regarding the down-regulated proteins of inverted karyotype cells, the GAPDH protein was again highlighted as the bottleneck in the network, with the highest betweenness-degree value in the centrality measurement graph (Figure 7c, 7d). This result indicates that GAPDH is the hub.

![Image](https://example.com/image.png)

**Figure 7.** a. Interaction network of down-regulated proteins in normal karyotype cells. b. Measurement of centrality of down-regulated proteins in normal karyotype cells. c. Interaction network of down-regulated proteins in inverted karyotype cells. d. Measurement of centrality of down-regulated proteins in inverted karyotype cells. Thick bars represent strong interactions. Thin bars represent weak interactions.

**Discussion**

This paper analyzed the expression profile of human MSC with normal and inverted (inv(3)(p25p13) karyotypes and pointed out some biological processes and molecular functions of these cell proteins. Bryukhovetskiy et al. (2014) performed proteomics using cells from bone marrow and nerve tissue. They found 607 stem cell proteins from bone marrow and 1052 stem cell proteins from nerve tissue. They used two different spectrometers with different ionization sources in the experiments; this fact probably justifies a large number of proteins found, since different spectrometers have a higher resolution power (Angelucci et al., 2010).

Firstly, normal karyotype cells had more BP and MF when compared to inverted karyotype cells (p < 0.05) (Figure 2). The cytoskeleton organization (the main biological process found in inverted karyotype cell proteins) and the cytoskeleton constitution (the main molecular function found in inverted karyotype cell proteins) can indicate changes in gene expression required for stem cells to give origin to a different cell line. For instance, the Direct Trans-Differentiation happens when the cell changes its cytoskeleton and its protein synthesis in order to differentiate itself in another specific cell type (Monteiro, Argolo Neto, & Del Carlo, 2010).

We also found the squamous cell carcinoma antigen 1 (SCC1) in inverted karyotype cells. SCC1 was super expressed in tumors, including the tongue, esophagus, uterine cervix, and skin tumors (Liu et al., 2015).
inverted karyotype cells may have differentiated into tumor cells due to inversion. Studies have reported chromosomal aberrations, immortalization, and malignant transformation in fresh MSC isolated from humans and rats after a considerable period of in vitro expansion (Duarte et al., 2012).

Regarding the normal karyotype cell proteins, the main biological process was cell transport. Proteins related to cell differentiation need to be transported to act on RNA (Tsai et al., 2015). This corroborates with the major molecular function of normal karyotype cell proteins (RNA binding). Several proteins bind to the stem cells’ RNA in order to increase the protein synthesis related to cell differentiation (Kwon et al., 2013). In addition, BPs related to cell regeneration and tissue injury were found in both cell types. However, the response to oxidative stress was only found in inverted karyotype cells, suggesting that these cells undergo greater oxidative stress when compared to normal karyotype cells.

In our study it was verified that KRT16, a keratin family member, was the bottleneck protein regarding the normal karyotype cell proteins. Keratins are subdivided into cytotkeratins and capillary keratins. Keratins are intermediate filaments of proteins responsible for the structural integrity of epithelial cells (Bragulla & Homberger, 2009), however the presence of KRT16 was due to sample contamination. Therefore, albumin is the bottleneck protein, with the second highest value of betweenness. Albumin is an intracellular and secreted plasma protein involved with the intracellular transport (biological process). This protein regulates the plasma colloid osmotic pressure and acts as a carrier protein for a wide range of endogenous molecules including hormones, fatty acids, and metabolites, as well as exogenous drugs (Naveen, Akshata, Pimple, & Chaudhari, 2016).

Albumin was associated with a positive prognosis in cancer. Pretreatment with serum albumin has useful significance in cancer. Accordingly, serum albumin level could be used in clinical trials to better define the baseline risk in cancer patients. However, a critical gap for demonstrating causality is the absence of clinical trials demonstrating that raising albumin levels by intravenous infusion or by hyperalimentation decreases the excess risk of mortality in cancer. Albumin was verified as being expressed in normal karyotype cells (Gupta & Lis, 2010).

DSP protein is also highlighted in the network. DSP is found in the cytoskeleton, desmosomes, and plasma membrane. DSP is involved in the organization of the desmosomal cadherin-plakoglobin complexes into discreet plasma membrane domains and in anchoring intermediate filaments to the desmosomes (https://www.uniprot.org/uniprot/P15924). PKP 1 protein found in the nucleus and in desmosomes is also very important in the network, playing a key role in junctional plaques and contributing to epidermal morphogenesis (https://www.uniprot.org/uniprot/Q13835).

RHOA was the bottleneck protein regarding the inverted karyotype cell proteins. RHOA is a member of the GTPase family and has been reported as regulating various biological activities, including the formation of stress fibers, gene transcription, membrane transport, and cell adhesion. RHOA is also related to cell survival and cell proliferation, and can therefore be related to cancer. RHOA was in fact super-expressed in inverted karyotype cells (Li, Chen, & Xu, 2011). Although RHOA has the highest betweenness degree, Figure 5c also highlights Serpine1 and CTSB. Interestingly, Serpine1 (https://www.proteinatlas.org/ENSG00000106366-SERPINE1/tissue) and CTSB (https://www.proteinatlas.org/ENSG00000164733-CTSB/tissue) genes are both related to cancer.

The analysis of up-regulated and down-regulated proteins of normal karyotype and inverted karyotype cells, respectively, showed that the bottleneck protein of both interaction networks is GAPDH. GAPDH is an enzyme which plays an important role in glycolysis. GAPDH catalyzes the phosphorylation of glyceraldehyde-3-phosphate into 1,3-bisphosphoglycerate in glucose metabolism using nicotinamide adenine dinucleotide (NAD) as a cofactor. Experimental evidence suggests that GAPDH is actually a multifunctional protein. GAPDH can regulate gene expression/transcription, has kinase/phosphotransferase activity, facilitates vesicular transport, and interacts with molecules, including ribozymes, glutathione (GSH), p53, and nitric oxide (El Kadmiri et al., 2014).

Although GAPDH has the highest betweenness degree, Figure 6a also highlights VCL. VCL binds to actin, is associated with the membrane and is found in the cell-cell and cell-matrix junctions. VCL has the ability to assemble the actin cytoskeleton and anchor it to the cell membrane through integrins (Zemljic-Harpf et al., 2014). This ability might suggest an adhesion activity between the stem cells, with each other and with the extracellular matrix. Defects in VCL are the cause of dilated cardiomyopathy (https://www.proteinatlas.org/ENSG0000035403-VCL/tissue).
In analyzing the up-regulated proteins of inverted karyotype cells, CCT3 and DECR1 were characterized as the bottleneck proteins. CCT3 is a protein subunit included in the chaperonins group, and present in eukaryotic cells. Chaperonins use ATP hydrolysis energy to increase the efficiency of the reactions, helping other proteins reach their functional conformations. The group of chaperonins in which the CCT3 is included has roles in actin and tubulin folding. These chaperonins also regulate molecules responsible for cell division and cytoskeletal regulatory proteins (Nadler-Holly et al., 2012). The bottleneck protein DECR1 is an enzyme involved in the auxiliary pathway of fatty acid oxidation. DECR1 limits the rate of a process which prepares polyunsaturated fatty acids to be used as substrates for beta-oxidation (Ursini-Siegel et al., 2007).

Finally, we found that UBC was the bottleneck protein among the down-regulated proteins of normal karyotype cells. Literature indicates that the UBC gene is required for extra ubiquitin synthesis during oxidative stress, and ubiquitin is able to remove damaged proteins. The loss of UBC gene function cannot be compensated by UBC gene induction. A decrease in ubiquitin levels leads to a decrease in the destruction of non-functional or defective proteins due to oxidative stress. We found low UBC expression in normal karyotype cells, and therefore we can suggest that these cells are not susceptible to high levels of oxidative stress (Crinelli et al., 2015). Overwall, these data may contribute to studies aiming toward genetic stability analyses of human MSC.

Conclusion

The results suggest that the paracentric inversion inv(3)(p25p13) induced cellular stress in human MSC, since proteins related to stress response were super expressed in inverted karyotype cells. In addition, we found the presence of squamous cell carcinoma antigen 1, Serpine 1, RHOA, and CTSB in inverted karyotype cells. Therefore, we can suggest that the inversion can contribute to genetic instability since these proteins are related to cancer. Albumin is related to a positive prognosis in cancer, and was super-expressed in normal karyotype cells. Finally, the low Ubiquitin levels in normal karyotype cells, possibly indicating low oxidative stress in these cells.

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**SUPPLEMENTARY MATERIAL**

**Table S1.** Proteins of cells with inverted karyotype. Data were generated with Scaffold software.

| Protein                                      | Accession Number | IK Spectra counta | NK Spectra countb | Fold Changec |
|----------------------------------------------|------------------|-------------------|-------------------|--------------|
| Retinal Dehydrogenase 1                     | 21361176         | 20                | 0                 | 0            |
| Squamous Cell Carcinoma Antigen-1           | 195792580        | 14                | 0                 | 0            |
| Keratin 16                                   | 1195551          | 28                | 0                 | 0            |
| Keratin 14                                   | 12803709         | 24                | 0                 | 0            |
| Fam1L29                                      | 32425737         | 2                 | 0                 | 0            |
| Desmoplakin I                                | 1147813          | 8                 | 0                 | 0            |
| Heat Shock                                   | 119582699        | 7                 | 0                 | 0            |
| Hpa28                                        | 1008915          | 1                 | 0                 | 0            |
| Threonine-Protein                           | 114576744        | 2                 | 0                 | 0            |
| Ras-Gtpase-Activating Protein               | 119582065        | 4                 | 0                 | 0            |
| Pro2619                                      | 11493459         | 4                 | 0                 | 0            |
| JUP Protein                                  | 15080189         | 5                 | 0                 | 0            |
| Calmodulin-Like Skin Protein C Terminal Domain | 109157166     | 5                 | 0                 | 0            |
| Band-6-Protein                               | 555015           | 2                 | 0                 | 0            |
| Metalloproteinases-3                         | 1304484          | 2                 | 0                 | 0            |

*a: IK: Inverted karyotype; b: NK: Normal karyotype; c: Fold Change ‘0’ means that there were no differences in the proteins karyotypes expressions.

**Table S2.** Proteins of cells with normal karyotype. Data were generated with Scaffold software.

| Protein name                                    | Accession Number | IK Spectra counta | NK Spectra countb | Fold Change |
|------------------------------------------------|------------------|-------------------|-------------------|-------------|
| NME1-NME2 Protein                              | 66392203         | 0                 | 6                 | 0           |
| Procathepin B At 5.2                           | 157833437        | 0                 | 6                 | 0           |
| Plasminogen Activator Inhibitor 1              | 10835159         | 0                 | 4                 | 0           |
| Arginine                                       | 119624305        | 0                 | 3                 | 0           |
| Unnamed Protein Product                         | 194388452        | 0                 | 3                 | 0           |
| CAPNS1 Protein                                 | 15080279         | 0                 | 3                 | 0           |
| HYOU1 Protein                                  | 116283359        | 0                 | 2                 | 0           |
| Unnamed Protein Product                         | 189065537        | 0                 | 3                 | 0           |
| ATP Synthase                                    | 51479152         | 0                 | 3                 | 0           |
| Neprilysin                                      | 116256327        | 0                 | 6                 | 0           |
| 26S Protease Regulatory Subunit 6B Isoform 2   | 24430155         | 0                 | 3                 | 0           |
| Pyrroline5-Carboxylate Reductase                | 114794869        | 0                 | 5                 | 0           |
| Reticulon 4                                     | 119620534        | 0                 | 5                 | 0           |
| Tat-Associated Protein                         | 1096067          | 0                 | 3                 | 0           |
| Ahnak                                          | 61745954         | 0                 | 6                 | 0           |
| 40S Ribosomal Protein S17                     | 4506693          | 0                 | 4                 | 0           |
| Recname                                        | 145811408        | 0                 | 2                 | 0           |
| Dihydrolipoamide S-Acetyltransferase           | 119587578        | 0                 | 3                 | 0           |
| Physiological Dimer Hpp Precursor              | 2098347          | 0                 | 3                 | 0           |
| Leucine                                        | 119579268        | 0                 | 3                 | 0           |
| Ribosomal Protein L3                           | 119580717        | 0                 | 5                 | 0           |
| Poly(Rc)-Binding Protein                      | 14141166         | 0                 | 1                 | 0           |
| Unnamed Protein Product                        | 194575854        | 0                 | 3                 | 0           |
| Cytochrome B-C1 L                             | 163644521        | 0                 | 1                 | 0           |
| Glycosyltransferase                           | 119605027        | 0                 | 1                 | 0           |
| Cyclooxygenase2                                | 181254           | 0                 | 3                 | 0           |
| Hydroxacyl-Coenzyme A Dehydrogenase           | 119621106        | 0                 | 3                 | 0           |
| GDP Dissociation Inhibitor 2                  | 119606856        | 0                 | 1                 | 0           |
| Guanine Nucleotide-Binding Protein            | 11055998         | 0                 | 2                 | 0           |
| Hgc2016877                                    | 119594653        | 0                 | 1                 | 0           |
| Unnamed Protein Product                        | 189065417        | 0                 | 2                 | 0           |
| Cysteine and Glycine-Rich Protein 2           | 4503101          | 0                 | 2                 | 0           |
| Proteasome                                     | 195539356        | 0                 | 1                 | 0           |
| Hgc39912                                      | 119576757        | 0                 | 1                 | 0           |
| Glycoprotein 1                                 | 112380628        | 0                 | 2                 | 0           |
| Glutaminase                                    | 114582297        | 0                 | 2                 | 0           |
| Aconitase 2                                   | 1232280108       | 0                 | 3                 | 0           |
| Unnamed Protein Product                        | 193786545        | 0                 | 2                 | 0           |
| Rivb                                           | 119599729        | 0                 | 1                 | 0           |
| SUB1 Homolog                                   | 16307067         | 0                 | 1                 | 0           |
| Ubiquinone                                     | 115387094        | 0                 | 2                 | 0           |
### Table S3. Proteins of cells with inverted and normal karyotype. Data were generated with Scaffold software.

| Protein name                  | Accession Number | IK Spectra count | NK Spectra count | Fold Change |
|-------------------------------|------------------|------------------|------------------|-------------|
| RNA Binding Protein           | 119626277        | 0                | 2                | 0           |

*IK: Inverted karyotype; *NK: Normal karyotype; 'Fold Change '0' means that there were no differences in the proteins karyotypes expressions.

| Protein name                  | Accession Number | IK Spectra count | NK Spectra count | Fold Change |
|-------------------------------|------------------|------------------|------------------|-------------|
| Ribonucleoprotein M           | 119589527        | 1                | 15               | 0.08        |
| Procollagen-Lysine 1          | 16741721         | 1                | 9                | 0.1         |
| Superoxide Dismutase          | 110590806        | 2                | 16               | 0.1         |
| Rab5C                         | 41593545         | 1                | 8                | 0.2         |
| Glucosidase                   | 11959451         | 5                | 30               | 0.2         |
| Plectin                       | 1477646          | 1                | 7                | 0.2         |
| Unnamed Protein Product       | 158254970        | 1                | 7                | 0.2         |
| Cytochrome B5                 | 119595674        | 1                | 7                | 0.2         |
| Proteasome                    | 54696300         | 1                | 6                | 0.2         |
| Rab1B                         | 14249144         | 1                | 6                | 0.2         |
| Unnamed Protein Product       | 194381290        | 8                | 41               | 0.2         |
| Unnamed Protein Product       | 158255578        | 2                | 10               | 0.2         |
| Ribosomal Protein L9          | 119613552        | 1                | 5                | 0.2         |
| Transmembrane Emp24           | 119605573        | 1                | 5                | 0.2         |
| Hmtdsb                        | 126030507        | 1                | 5                | 0.2         |
| SYNCRIP Protein               | 116283697        | 2                | 9                | 0.5         |
| Unnamed Protein Product       | 158261055        | 1                | 4                | 0.5         |
| Prohibitin 2                  | 119609105        | 2                | 9                | 0.5         |
| Fibronec1                     | 119590945        | 64               | 224              | 0.5         |
| Rab7                          | 1174149          | 5                | 16               | 0.5         |
| Trifunctional Enzyme          | 20127408         | 4                | 12               | 0.5         |
| Chaperonin Containing TCP1    | 14124984         | 4                | 12               | 0.5         |
| Unnamed Protein Product       | 37138            | 6                | 20               | 0.5         |
| Splicing Factor Proline       | 119627830        | 2                | 8                | 0.5         |
| Ribosomal Protein S19         | 16924231         | 4                | 11               | 0.5         |
| Unknown                       | 12804225         | 5                | 14               | 0.4         |
| Matri-3                      | 21626466         | 4                | 10               | 0.4         |
| Hcg1994130                   | 119570641        | 4                | 10               | 0.4         |
| Electron Transfer Flavoprotein| 189181759        | 2                | 7                | 0.4         |
| Ribonucleoproteins C1 C2 1-1-Like | 109082737    | 4                | 10               | 0.4         |
| Epb72                         | 119578798        | 1                | 3                | 0.4         |
| Peroxisoroxin 4              | 149245259        | 1                | 5                | 0.4         |
| 2,4-Dienoyl-Coa Reductase     | 157500           | 1                | 3                | 0.4         |
| Proteasome                    | 4506181          | 1                | 3                | 0.4         |
| Polypyrimidine                | 119581557        | 6                | 16               | 0.4         |
| Ribonucleoproteins B1         | 14043072         | 5                | 13               | 0.4         |
| Glycyl-Trna Synthetase        | 116805540        | 4                | 9                | 0.4         |
| Calreticulin                  | 119604736        | 10               | 25               | 0.4         |
| Myosin-1c                     | 124494238        | 2                | 6                | 0.4         |
| Hcg2043289                   | 119575627        | 2                | 6                | 0.4         |
| Hcg1640785                   | 119569529        | 2                | 6                | 0.4         |
| Unnamed Protein Product       | 158256826        | 8                | 21               | 0.4         |
| Ribosomal Protein S24         | 119575003        | 6                | 14               | 0.4         |
| Unnamed Protein Product       | 189054446        | 6                | 14               | 0.4         |
| Hcg1745306                   | 119606268        | 2                | 5                | 0.5         |
| Proteasome                    | 119567805        | 2                | 5                | 0.5         |
| Nucleosome Structure          | 296863426        | 1                | 3                | 0.5         |
| Signal Recognition Particle   | 197099116        | 2                | 5                | 0.5         |
| SH3                          | 119628236        | 1                | 3                | 0.5         |
| Transferrin Receptor          | 119574056        | 1                | 3                | 0.5         |
| Endoplasmin Precursor         | 4507677          | 31               | 67               | 0.5         |
| Mitochondrial Precursor       | 4502303          | 4                | 8                | 0.5         |
| 40S Ribosomal Protein S12     | 14277700         | 4                | 8                | 0.5         |
| Unnamed Protein Product       | 189055102        | 10               | 20               | 0.5         |
| 2-Oxoglutarate 5-Dioxygenase 2| 119599546        | 6                | 12               | 0.5         |
| Protein Disulfide             | 20070125         | 23               | 45               | 0.5         |
| Lamin A                       | 119573581        | 38               | 76               | 0.5         |
| Voltage-Dependent Anion Channel 2| 11974954       | 10               | 19               | 0.5         |

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| Protein name                      | Accession Number | IK Spectra count | NK Spectra count | Fold Changea |
|----------------------------------|------------------|------------------|------------------|--------------|
| Human Galectin1                  | 42542977         | 28               | 54               | 0.5          |
| X-Ray                            | 169145200        | 4                | 7                | 0.5          |
| 5′-Phosphoadenosine 5′-Phosphosulfate Synthase 2 | 119570566       | 4                | 7                | 0.5          |
| T-Plasmin Polypeptide            | 190028           | 10               | 18               | 0.5          |
| Heat Shock Protein               | 153792590        | 25               | 47               | 0.5          |
| Ribosomal Protein                | 337518           | 6                | 11               | 0.5          |
| Histone H2A Type 1-B E 1        | 10645195         | 19               | 54               | 0.6          |
| Ribosomal Protein S18            | 119624101        | 7                | 13               | 0.6          |
| Chain B                          | 157879202        | 5                | 9                | 0.6          |
| Hcg2103189                      | 119603728        | 2                | 4                | 0.6          |
| Unnamed Protein Product          | 194580796        | 2                | 4                | 0.6          |
| Far Upstream Element             | 11962762         | 2                | 4                | 0.6          |
| Unnamed Protein Product          | 194375608        | 2                | 4                | 0.6          |
| Tapasinerp57                    | 220702506        | 26               | 46               | 0.6          |
| Ubiquitin                        | 23510338         | 11               | 19               | 0.6          |
| Mitochondrial F1 Complex         | 127798841        | 11               | 19               | 0.6          |
| Glucose-Regulated Protein        | 16507257         | 59               | 99               | 0.6          |
| Initiation Factor Eif5a          | 183448588        | 4                | 6                | 0.6          |
| Rab18                            | 10880989         | 4                | 6                | 0.6          |
| Heat Shock                       | 12653415         | 26               | 44               | 0.6          |
| ATP-Dependent RNA Helicase DDX17 | 148613856        | 5                | 8                | 0.6          |
| Tropomyosin Beta                 | 47519616         | 30               | 48               | 0.6          |
| Unnamed Protein Product          | 194586896        | 32               | 51               | 0.6          |
| Protein L13                      | 15431295         | 6                | 9                | 0.6          |
| Mitochondrial Precursor          | 32189394         | 48               | 75               | 0.6          |
| Calnexin Precursor               | 10716563         | 17               | 26               | 0.7          |
| Cytoskeleton-Associated Protein 4 | 19263767         | 58               | 57               | 0.7          |
| Tropomyosin Alpha1               | 27597085         | 26               | 39               | 0.7          |
| Alpha1(E)-Catenin                | 1172426          | 5                | 7                | 0.7          |
| Phosphoprotein P1                | 31979223         | 5                | 7                | 0.7          |
| L18a                             | 11415026         | 2                | 3                | 0.7          |
| PSMA7 Protein                    | 116283481        | 4                | 5                | 0.7          |
| Myoferlin                        | 119570458        | 2                | 3                | 0.7          |
| Fructose-Bisphosphatase          | 119600542        | 1                | 2                | 0.7          |
| Spectrin                         | 112382250        | 2                | 3                | 0.7          |
| Unnamed Protein Product          | 189065399        | 1                | 2                | 0.7          |
| Sec23A                           | 109083408        | 2                | 3                | 0.7          |
| Unnamed Protein Product          | 189053683        | 2                | 3                | 0.7          |
| Protein 1                        | 141797011        | 1                | 2                | 0.7          |
| Unnamed Protein Product          | 140420358        | 1                | 2                | 0.7          |
| Hcg2028724                       | 119601423        | 8                | 12               | 0.7          |
| Hcg21078                         | 119568094        | 6                | 9                | 0.7          |
| Drebrin 1                        | 119605595        | 6                | 9                | 0.7          |
| Protein 5                        | 1710248          | 29               | 40               | 0.7          |
| Ribosomal Protein SA             | 119584991        | 8                | 10               | 0.8          |
| Ribosomal Protein S8             | 119627428        | 12               | 15               | 0.8          |
| Heat Shock Protein               | 4504517          | 36               | 44               | 0.8          |
| Cyclosporin                      | 1310882          | 28               | 33               | 0.8          |
| Actin Related Protein 2 3′-5′ Complex | 119618519    | 4                | 4                | 0.8          |
| Hexokinase 1                     | 119574708        | 7                | 9                | 0.8          |
| Unnamed Protein Product          | 194582308        | 4                | 4                | 0.8          |
| Glycophorin 2                    | 169790853        | 4                | 4                | 0.8          |
| Alpha–Actinin4                   | 12025678         | 108              | 128              | 0.8          |
| Prolin I                         | 15783469         | 20               | 24               | 0.9          |
| Unnamed Protein Product          | 194580758        | 13               | 15               | 0.9          |
| Ribosomal Protein S16            | 119577296        | 13               | 15               | 0.9          |
| Malate Dehydrogenase             | 21735621         | 19               | 22               | 0.9          |
| Major Vault Protein              | 19913410         | 25               | 29               | 0.9          |
| Alpha–Actinin1                   | 194097350        | 160              | 184              | 0.9          |
| Non-Erythrocytic 1               | 119608213        | 6                | 7                | 0.9          |
| Ribosomal Protein L30            | 119612175        | 8                | 9                | 0.9          |
| Ribosomal Protein SA             | 119584991        | 8                | 10               | 0.8          |
| Ribosomal Protein S8             | 119627428        | 12               | 15               | 0.8          |
| Heat Shock Protein               | 4504517          | 36               | 44               | 0.8          |
| Cyclosporin                      | 1310882          | 28               | 33               | 0.8          |
| Protein name                      | Accession Number | IK Spectra count | NK Spectra count | Fold Change | Comment |
|----------------------------------|------------------|------------------|------------------|-------------|---------|
| Actin Related Protein 2 5' Complex| 119618519        | 4                | 4                | 0.8         |         |
| Hexokinase I                     | 11957408        | 7                | 9                | 0.8         |         |
| Unnamed Protein Product          | 1943828308       | 4                | 4                | 0.8         |         |
| Glycoprotein 2                   | 169790853       | 4                | 4                | 0.8         |         |
| Alpha-Actinin4                   | 12025678        | 108              | 128              | 0.8         |         |
| Profilin I                       | 157833469       | 20               | 24               | 0.9         |         |
| Unnamed Protein Product          | 194380758       | 13               | 15               | 0.9         |         |
| Ribosomal Protein S16            | 119577296       | 13               | 15               | 0.9         |         |
| Malate Dehydrogenase             | 21755621        | 19               | 22               | 0.9         |         |
| Major Vault Protein              | 19913410        | 25               | 29               | 0.9         |         |
| Alpha-Actinin1                   | 194097550       | 160              | 184              | 0.9         |         |
| Non-Erythrocytic 1               | 119608215       | 6                | 7                | 0.9         |         |
| Ribosomal Protein L30            | 119612175       | 8                | 9                | 0.9         |         |
| Unnamed Protein Product          | 1943828308       | 4                | 4                | 0.8         |         |
| Glycoprotein 2                   | 169790853       | 4                | 4                | 0.8         |         |
| Alpha-Actinin4                   | 12025678        | 108              | 128              | 0.8         |         |
| Profilin I                       | 157833469       | 20               | 24               | 0.9         |         |
| Unnamed Protein Product          | 194380758       | 13               | 15               | 0.9         |         |
| Ribosomal Protein S16            | 119577296       | 13               | 15               | 0.9         |         |
| Malate Dehydrogenase             | 21755621        | 19               | 22               | 0.9         |         |
| Major Vault Protein              | 19913410        | 25               | 29               | 0.9         |         |
| Alpha-Actinin1                   | 194097550       | 160              | 184              | 0.9         |         |
| Non-Erythrocytic 1               | 119608215       | 6                | 7                | 0.9         |         |
| Ribosomal Protein L30            | 119612175       | 8                | 9                | 0.9         |         |
| Unnamed Protein Product          | 158254664       | 122              | 137              | 0.9         |         |
| Rab1a                            | 119620529       | 19               | 21               | 0.9         |         |
| Heat Shock Cognate               | 5729877         | 88               | 95               | 0.9         |         |
| Histone 1                        | 119575932       | 35               | 38               | 0.9         |         |
| Nucleosomin                      | 10835063        | 19               | 21               | 0.9         |         |
| Peroxiredoxin6                   | 4758638         | 12               | 15               | 0.9         |         |
| Ribosomal Protein L18            | 119572744       | 10               | 10               | 0.9         |         |
| 40S Ribosomal Protein S14        | 5032051         | 2                | 3                | 0.9         |         |
| WD Repeat Domain 1               | 119613095       | 2                | 3                | 0.9         |         |
| Strongylocentrotus Purpuratus    | 119607750       | 2                | 3                | 0.9         |         |
| EIF5A Protein                    | 116283747       | 2                | 3                | 0.9         |         |
| Unnamed Protein Product          | 193783525       | 2                | 3                | 0.9         |         |
| Unnamed Protein Product          | 31092           | 54               | 56               | 0.9         |         |
| Vdac1                            | 198443050       | 17               | 18               | 0.9         |         |
| 60S Acidic Ribosomal Protein P2  | 4506671         | 14               | 15               | 0.9         |         |
| 40S Ribosomal Protein S9         | 14141193        | 7                | 8                | 0.9         |         |
| Dehydrogenase                    | 15786847        | 7                | 8                | 0.9         |         |
| MYL6 Protein                     | 113812151       | 23               | 24               | 1           |         |
| 60S Ribosomal Protein L12        | 4506597         | 15               | 14               | 1           |         |
| Serpin H1 Precursor              | 32454741        | 53               | 54               | 1           |         |
| 40S Ribosomal Protein            | 114647215       | 8                | 9                | 1           |         |
| Hcg1983058                       | 119619456       | 8                | 9                | 1           |         |
| Rap-Rappag                      | 169791854       | 6                | 6                | 1           |         |
| Rab2A                            | 356391095       | 6                | 6                | 1           |         |
| Triosephosphate Isomerase 1      | 17389815        | 18               | 18               | 1           |         |
| Ribonucleoprotein A1             | 14043070        | 18               | 18               | 1           |         |
| Ribonucleoprotein K              | 119583080       | 16               | 15               | 1           |         |
| Ribosomal Protein S10            | 119624187       | 10               | 9                | 1           |         |
| Pyruvate Kinase                  | 119582922       | 46               | 45               | 1           |         |
| ADP-Ribosylation Factor 4        | 4502205         | 13               | 13               | 1           |         |
| Protein name                                      | Accession Number | IK Spectra count | NK Spectra count | Fold Change |
|--------------------------------------------------|------------------|------------------|------------------|-------------|
| Ribosomal Protein S5                             | 119592989        | 13               | 13               | 1           |
| Hsp70 Atpase                                     | 166007012        | 13               | 13               | 1           |
| Actin                                            | 4501887          | 275              | 267              | 1           |
| Elongation Factor 2                              | 4505483          | 83               | 81               | 1           |
| Histone H2B                                      | 10800138         | 59               | 57               | 1           |
| Tubulin Beta6                                     | 27754056         | 61               | 58               | 1.1         |
| Serpin H1 Precursor                              | 32454741         | 53               | 54               | 1           |
| 40S Ribosomal Protein                            | 11467215         | 8                | 9                | 1           |
| Hcg1983058                                      | 119619456        | 8                | 9                | 1           |
| Rap-Rapgap                                       | 169791854        | 6                | 6                | 1           |
| Rab2A                                            | 326391093        | 6                | 6                | 1           |
| Triosephosphate Isomerase 1                      | 17389815         | 18               | 18               | 1           |
| Ribonucleoprotein A1                             | 14043070         | 18               | 18               | 1           |
| Ribonucleoprotein K                              | 119583080        | 16               | 15               | 1           |
| Ribosomal Protein S10                            | 119624187        | 10               | 9                | 1           |
| Pyruvate Kinase                                  | 119598292        | 46               | 45               | 1           |
| ADP-Ribosylation Factor 4                        | 4502205          | 13               | 13               | 1           |
| Ribosomal Protein S5                             | 119592989        | 13               | 13               | 1           |
| Hsp70 Atpase                                     | 166007012        | 13               | 13               | 1           |
| Actin                                            | 4501887          | 275              | 267              | 1           |
| Elongation Factor 2                              | 4505483          | 83               | 81               | 1           |
| Histone H2B                                      | 10800138         | 59               | 57               | 1           |
| Tubulin Beta6                                     | 27754056         | 61               | 58               | 1.1         |
| Protein-Dissulfide                               | 192987144        | 4                | 3                | 1.1         |
| Adaptor-Related Protein Complex 1                | 119580203        | 4                | 3                | 1.1         |
| Histone H2B                                      | 10800140         | 60               | 57               | 1.1         |
| Ran-Like                                         | 109099257        | 8                | 8                | 1.1         |
| Tubulin                                          | 119608775        | 114              | 105              | 1.1         |
| Importin                                         | 119615215        | 18               | 16               | 1.1         |
| Beta-Tubulin                                     | 1297274          | 88               | 79               | 1.1         |
| Phosphoglycerate Mutase 1                        | 297302419        | 5                | 4                | 1.1         |
| Filamin-B                                        | 105990514        | 30               | 27               | 1.1         |
| Keratin 8                                        | 119617057        | 108              | 95               | 1.2         |
| Tubulin                                          | 18088719         | 140              | 121              | 1.2         |
| Glyceraldehyde-5-Phosphate Dehydrogenase         | 31645            | 78               | 67               | 1.2         |
| Vinculin                                         | 24657579         | 30               | 26               | 1.2         |
| Ribonucleoprotein U                              | 14141161         | 12               | 10               | 1.2         |
| T-Complex Protein 1                              | 261599877        | 6                | 5                | 1.2         |
| Chaperonin                                       | 119620590        | 6                | 5                | 1.2         |
| Calelecin                                        | 179976           | 49               | 41               | 1.2         |
| Myosin                                          | 15809016         | 26               | 21               | 1.2         |
| Chain A                                         | 157831404        | 62               | 51               | 1.2         |
| Translocon-Associated Protein Subunit Delta Isofor 1 Precursor | 325301072 | 11               | 9                | 1.5         |
| Keratin. Type I Cytoskeletal 19                  | 24254699         | 23               | 18               | 1.3         |
| Adp-Ribosylation Factor 1                        | 1065361          | 12               | 9                | 1.3         |
| G Protein                                       | 119574079        | 56               | 28               | 1.5         |
| Moesin.                                         | 119625804        | 25               | 20               | 1.5         |
| 14-3-3 Protein Epsilon                           | 114665591        | 42               | 33               | 1.5         |
| Unnamed Protein Product                          | 193786502        | 90               | 69               | 1.5         |
| Cofilin1                                        | 5031635          | 44               | 33               | 1.5         |
| KIAA1027 Protein                                | 20521736         | 68               | 51               | 1.3         |
| Keratin 18                                      | 12653819         | 24               | 18               | 1.3         |
| Glutathione Transferase P11                     | 11514451         | 22               | 15               | 1.4         |
| Ribosomal Protein L25a                          | 119571516        | 11               | 8                | 1.4         |
| Ubiquitin Thiolesterase                         | 119613588        | 10               | 7                | 1.4         |
| Hcg37214                                        | 119622042        | 10               | 7                | 1.4         |
| Zyxin                                           | 119572235        | 7                | 5                | 1.4         |
| Cytoplasmic 1                                   | 119612225        | 5                | 3                | 1.4         |
| Chain A                                         | 157831404        | 62               | 51               | 1.2         |
| Translocon-Associated Protein Subunit Delta Isofor 1 Precursor | 325301072 | 11               | 9                | 1.5         |
| Keratin. Type I Cytoskeletal 19                  | 24254699         | 23               | 18               | 1.3         |
| Adp-Ribosylation Factor 1                        | 1065361          | 12               | 9                | 1.3         |
| G Protein                                       | 119574079        | 36               | 28               | 1.5         |
| Moesin.                                         | 119625804        | 25               | 20               | 1.3         |
| 14-3-3 Protein Epsilon                           | 114665591        | 42               | 33               | 1.3         |
| Protein name | Accession Number | IK Spectra count | NK Spectra count | Fold Change |
|--------------|------------------|------------------|------------------|-------------|
| Unnamed Protein Product | 193786502 | 90 | 69 | 1.3 |
| Cofilin1 | 5031655 | 44 | 33 | 1.3 |
| KIAA1027 Protein | 20521736 | 68 | 51 | 1.3 |
| Keratin 18 | 12653819 | 24 | 18 | 1.3 |
| Glutathione Transferase P11 | 11514451 | 22 | 15 | 1.4 |
| Ribosomal Protein L25a | 119571516 | 11 | 8 | 1.4 |
| Ubiquitin Thiolesterase | 11961388 | 10 | 7 | 1.4 |
| Hgc57214 | 119622042 | 10 | 7 | 1.4 |
| Zyxin | 119572233 | 7 | 5 | 1.4 |
| Cytoplasmic 1 | 119612225 | 5 | 3 | 1.4 |
| Hgc35299 | 119597983 | 2 | 2 | 1.4 |
| Hgc198570 | 11960462 | 1 | 1 | 1.4 |
| Unnamed Protein Product | 221045576 | 4 | 3 | 1.4 |
| Ribosomal Protein L10 | 119595144 | 1 | 1 | 1.4 |
| Kh | 119627980 | 1 | 1 | 1.4 |
| C-Type Mannose Receptor 2 | 110624774 | 4 | 3 | 1.4 |
| Eukaryotic Translation Elongation Factor 1 Gamma | 15530265 | 5 | 3 | 1.4 |
| Flavoprotein | 119571567 | 2 | 2 | 1.4 |
| Coactosin-Like Protein | 21624607 | 2 | 2 | 1.4 |
| ATP Synthase | 16877071 | 4 | 3 | 1.4 |
| Protein B | 181486 | 1 | 1 | 1.4 |
| 26S Proteasome Subunit P97 | 1060888 | 1 | 1 | 1.4 |
| Integrin Beta 4 Binding Protein | 119596626 | 1 | 1 | 1.4 |
| Ribonucleaseprotein H1 | 119574194 | 8 | 6 | 1.4 |
| Pyrroline 5-Carboxylate Synthetase | 1304514 | 6 | 4 | 1.4 |

Phosphorylation Independent Interactions Between 14-3-3

| Ribosomal Protein S4 | 16172138 | 62 | 45 | 1.5 |
| Unnamed Protein Product | 179599221 | 18 | 12 | 1.5 |
| Tsα | 194390460 | 14 | 9 | 1.5 |
| Myosin-9 | 1617118 | 13 | 9 | 1.5 |
| Tyrosine 5-Monoxygenase | 12667788 | 207 | 134 | 1.5 |
| Chloride Intracellular Channel Protein 1 | 14251209 | 12 | 8 | 1.6 |
| Tubulin Alpha1a | 6755901 | 84 | 54 | 1.6 |
| 14-3-3 Protein Beta Alpha 1 | 14507949 | 24 | 15 | 1.6 |
| Prohibitin | 46360168 | 11 | 7 | 1.6 |
| L-Lactate Dehydrogenase | 13786849 | 10 | 6 | 1.6 |
| Annexin A2 | 18645167 | 159 | 87 | 1.6 |
| Transgelin | 119587704 | 136 | 81 | 1.7 |
| Clathrin | 119614801 | 7 | 4 | 1.7 |
| Unnamed Protein Product | 189059323 | 7 | 4 | 1.7 |
| Calcium-Calmodulin | 16974825 | 11 | 6 | 1.8 |

Unnamed Protein Product | 193785841 | 51 | 17 | 1.8 |
| Peroxiredoxin 1 | 119627382 | 25 | 14 | 1.8 |
| Keratin 9 | 119581148 | 14 | 8 | 1.9 |
| Filamin-C | 116805522 | 50 | 26 | 2 |
| Transgelin2-Like | 297663020 | 64 | 32 | 2 |
| Hgc1783090 | 119582155 | 22 | 10 | 2.1 |

| Unnamed Protein Product | 194387966 | 4 | 2 | 2.1 |
| Unnamed Protein Product | 104852896 | 4 | 2 | 2.1 |
| Phosphatidylinositol Binding Clathrin Assembly Protein | 119595524 | 4 | 2 | 2.1 |
| Annexin A1 | 119582950 | 56 | 25 | 2.3 |
| Rhodli K115a | 14278162 | 6 | 3 | 2.3 |
| Unknown | 13097759 | 25 | 10 | 2.5 |
| Keratin | 47132620 | 56 | 21 | 2.7 |
| Unnamed Protein Product | 189054048 | 7 | 3 | 2.8 |
| Unknown | 1531594 | 7 | 3 | 2.8 |

Simlar To Cysteine And Glycine-Rich Protein 1

| Proteasome | 8594076 | 2 | 1 | 2.8 |
| Hnr RNA Binding Protein | 1022961 | 8 | 3 | 3.3 |
| Keratin 1 | 11950549 | 192 | 54 | 3.6 |
| Ribosomal Protein L14 | 17952938 | 10 | 3 | 3.7 |
| Recname | 1703319 | 5 | 1 | 5.6 |
| Hgc1995201 | 11958408 | 7 | 1 | 8.4 |
| Ed42hc | 145579147 | 7 | 1 | 8.4 |
| RAN Binding Protein 5 | 119629385 | 7 | 1 | 8.4 |

*IK Spectra count* | *NK Spectra count* | *Fold Change*
| Protein name       | Accession Number | IK Spectra count | NK Spectra count | Fold Change |
|--------------------|------------------|------------------|------------------|-------------|
| Keratin 10         | 119581085        | 161              | 11               | 14          |
| Filamin-C          | 116805522        | 50               | 26               | 2           |
| Transgelin2-Like   | 297663020        | 64               | 32               | 2           |
| Hrg1783090         | 119582155        | 22               | 10               | 2.1         |

*IK: Inverted karyotype; 'NK: Normal karyotype; 'Fold Change '0' means that there were no differences in the proteins karyotypes expressions.*