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Analysis of Glutathione S-Transferase Pi (GSTP1) Expression in Non Melanoma Skin Cancer using Bioinformatic Tools

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Abstract

A bioinformatics study was carried out to study the expression of GSTP1 in non melanoma skin cancer (actinic keratosis and squamous cell carcinoma) by selecting non melanoma skin cancer research (record number GDS 2200) from the NCBI site. The experiment was analyzed using GEO data set and expression of GSTP1 in both control and actinic keratotic (AK) lesion, and squamous cell carcinoma (SCC) tumor biopsies data from 5 patients with non-melanoma skin cancer (NMSC) were analyzed. Known interactions for GSTP1 and coexpressed genes were also carried out using STRING database. The result shows that GSTP1 was over-expressed in the non melanoma samples (actinic keratosis and squamous cell carcinoma) compare with the control. Although one sample in the actinic keratosis shows higher expression, all the samples in squamous cell carcinoma show high expression of GSTP1. Result from STRING data base show that GSTP1interact and co-expressed with a number of genes which are important in understanding of cancer and progression of the disease. From the data generated, we concluded that GSTP1 was over-expressed in non melanoma skin cancer.

Keywords: Analysis, glutathione, expression, skin cancer, Bioinformatic tools.

Introduction

Human GSTP1 has drawn considerable interest due to it's over expression in cancer cells and likely involvement in acquired resistance of chemotherapeutic agents. The multifunctional enzyme glutathione S-transferase pi (GSTP1) is an important enzyme belonging to the glutathione S-transferase (GST) family of enzymes that carries out detoxification thereby inactivating electrophonic carcinogens by conjugation with reduced glutathione[1,2]. The enzyme participates in detoxification reactions by catalyzing the conjugation of many electrophilic and hydrophobic compounds with reduced glutathione. The soluble GSTs are categorized into 4 main classes based on their biochemical, immunologic, and structural properties. These classes include alpha, mu, pi, and theta. Hypermethylation has been found to have an effect on the regulatory sequence closed to the GST gene during the early stages of carcinogenesis[3-6]. Several classes of GST were earlier been reported in tissues of human beings and are being classified as alpha, mu, pi, and theta. An increased expression of GST pi has been reported in cancers of the breast, bladder, pancreas, colon, stomach, lung, neck and head, cervix, ovary, and, as well as soft tissue sarcoma, testicular embryonal carcinoma, glioma and meningioma[7-16]. GSTP 1 functions in metabolism of xenobiotics and is engaged in susceptibility to cancer, and other diseases. Reports link increased expression of glutathione *S*-transferase (GST) family of enzymes to acquired resistance to antineoplastic drugs[17]. Particular GST subclasses expression protects cancer cells from the cytotoxicities of cancer drugs, and antineoplastic drug resistance has been connected to over-expression of GST[18]. Substrate specificity of GST enzymes is of extensive among which are known to possess mutagenic properties. Serum GST pi elevation has been developed and exploited as a marker for serum tumor gastrointestinal cancer[8] and non-Hodgkin's lymphoma[19] and also as a method of predicting chemotherapy sensitivity. GST plays distinct roles in resistance to chemotherapy drugs via direct detoxification as well as inhibition of the MAP kinase pathway. GSTs localized mainly in the cytosol and are dimeric in nature. In addition to their catalytic role in detoxification, the enzymes also have broad ligand binding properties[20]. The expression of GST-pi has been compared in several malignant and normal tissues[21] and the research suggest that expression of GST-pi increases in many tumors compared to normal tissues. GST3 has been demonstrated to be abundantly expressed in human skin[22]. The aim of this paper is investigate whether there is over-expression of GSTP1 in non melanoma skin cancer specifically actinic keratosis lesion and squamous cell carcinoma.

Methodology

Selecting the experiment: Suitable experiment was selected from NCBI GEO data set by pointing Internet browser at http://www.ncbi.nlm.nih.gov/gds. Skin cancer was entered into search option and experiment with the title "non melanoma skin cancer" was selected. The detail of the experiment appears. The record number of the experiment GDS 2200, was noted for further analysis. The normal, actinic keratosis and squamous cell carcinoma samples number was also noted (normal: GSM47616, GSM47617, GSM47618, GSM47619, GSM47620, and GSM47621; actinic keratosis: GSM47612, GSM47613, GSM47614, GSM47615 and squamous cell carcinoma:

GSM47622, GSM47623, GSM47624, GSM47625 and GSM47626).

Analysis of the experiment using NCBI Geo data set data analysis tools: The record GDS 2200 was clicked and the main page having experimental details and analysis tools appears. The gene of interest (GSTP1) was entered into "find gene name or symbol" and Go button was clicked. A new window having expression pattern image of GSTp1 in the experiment appears along with gene annotation details. The image was click to visualize the differential expression of GSTP1 in both normal, actinic keratosis and squamous cell carcinoma samples (figure 3). Genes that are expressed up/down in the disease state were also analyzed by clicking Go button on the data analysis tools. A new window having list of gene showing differential expression in this experiment appears along with expression pattern images and gene annotation details. The image below the cluster was clicked to find co-expressed genes.

Figure-1
Selecting experiment from NCBI site

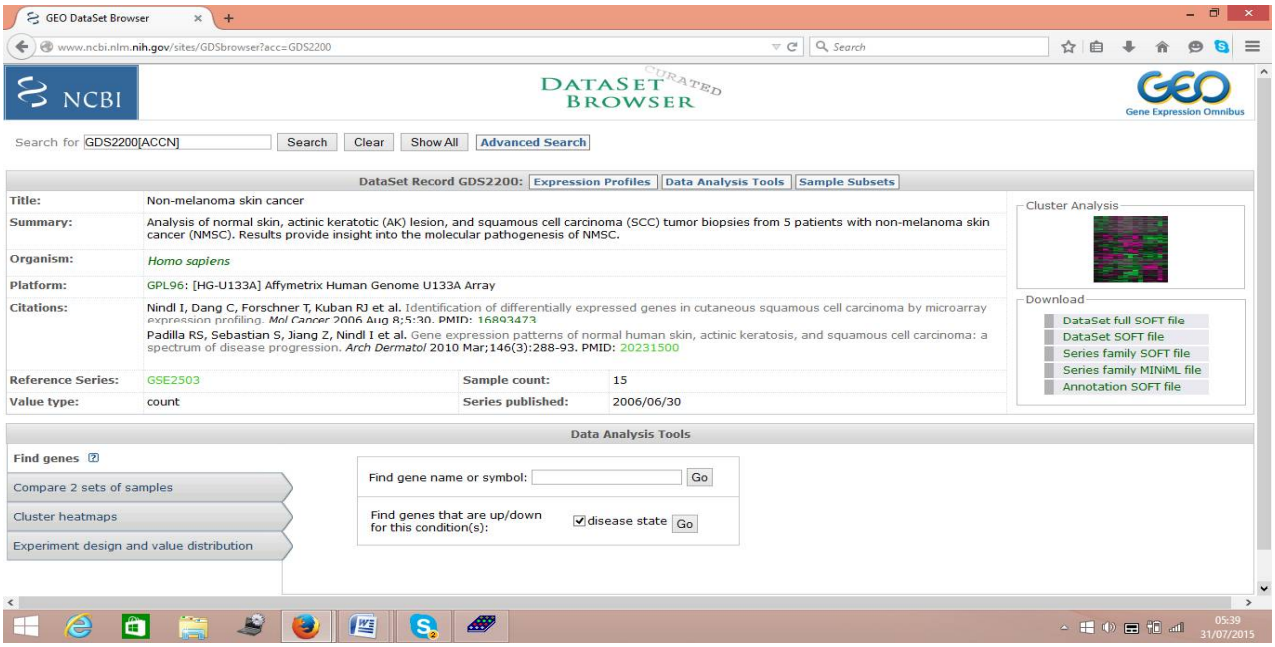
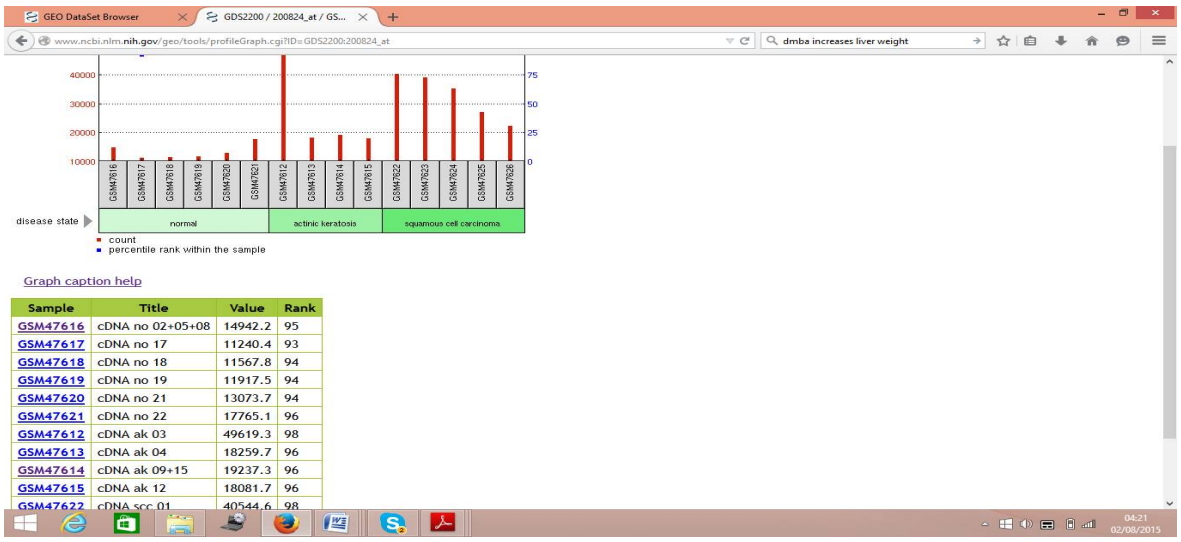


Figure-2
New window showing details of the selected experiment and data analysis tools



Sample	Title	Value	Rank
GSM47616	cDNA no 02+05+08	14942.2	95
GSM47617	cDNA no 17	11240.4	93
GSM47618	cDNA no 18	11567.8	94
GSM47619	cDNA no 19	11917.5	94
GSM47620	cDNA no 21	13073.7	94
GSM47621	cDNA no 22	17765.1	96
GSM47612	cDNA ak 03	49619.3	98
GSM47613	cDNA ak 04	18259.7	96
GSM47614	cDNA ak 09+15	19237.3	96
GSM47615	cDNA ak 12	18081.7	96
GSM47622	cDNA scc 01	40544.6	98

Figure 3
Visualizing the differential expression of GSTP1

Finding known interactions for GSTP1 and co-expressed genes using STRING database: The knowledge about interactions of GSTP1 and co-expressed genes will be helpful in understanding their role in cancer. The browser was pointed to STRING database at http://string-db.org/ (figure 4) GSP1 was searched into the protein name. Homo sapiens were selected as the organism's name. The interactions were finally analyzed.

Results and Discussion

The result of the analysis of glutathione s-transferase pi expression in non melanoma skin cancer using bioinformatics tools were display in figure-4. The original samples were collected from 5 patients and were Analysis for normal skin, actinic keratotic (AK) lesion, and squamous cell carcinoma (SCC) tumor biopsies from 5 patients with non-melanoma skin cancer (NMSC)[23].

The result shows that GSTP1 was over-expressed in the non melanoma samples (actinic keratosis and squamous cell carcinoma) compare with the control. Although one sample in the actinic keratosis shows higher expression, all the samples in squamous cell carcinoma show high expression of GSTP1. GSTP1 has become a subject of particular interest with regard to cancer, because many cancer cell lines and tumors are characterized by high GSTP1 expression[24]. it gene is encoded by a single gene has been mapped to chromosome 11q13. The mechanism by which GSH carried out detoxification involves nucleophilic attack on the sulphur atom of GSH onto the electrophilic group of many foreign substances and detoxification of anticancer drugs occurs not only by acting directly on the molecules but rather on a metabolite[25]. This action of GSTP1 significantly decreases the reactivity of these xenobiotics and increases their solubility favoring their elimination. In view of these, GST enzymatic activity may play a key role in the detoxification of a variety of anticancer drugs[25,26]. Over-expression of GSTP1 was observed in nearly all of human tumor cell lines, including those that are chemotherapeutic resistance[27,28]. A varieties of human cancers such as kidney; colon, breast, ovarian and lung usually express high levels of GSTP1 in comparison to the surrounding tissues. Accordingly, GSTP1-1 expression has been explored to be a marker for cancer development[29,30]. High expression levels have been correlated not only with drug resistance in patients undergoing chemotherapy but also with disease progression.

Analysis of the known and predicted interactions of GSTP1 have indicated that GSTP1 interact with a number of genes CPY1B1, CPY1A1, MAPK8, GSTB2B, EPHX1, GSST1, PRDX6, GSTO1, GSTT1, CYP2E1 and CYP1A2 (figure-6). The knowledge about interactions of GSTP1 and co-expressed genes will be helpful in understanding their role in cancer as well as progression of the disease. Association between several isoenzymes from GST classes with mitogen-activated protein kinase (MAPK) has been well established. Mitogen-activated protein kinase (MAPK) is involved in cell death signaling and cell survival and GSTP1 has been implicated in inhibition of this pathway thereby favoring survival of cancer cells. GSTs function non-enzymatically by sequestering the kinase in a complex, therefore preventing it from acting on downstream targets, consequently regulating pathways that control apoptotic cell death and cell proliferation. GST P1 was found to inhibit c-Jun N-terminal kinase (JNK) through direct protein–protein inter-action[31]. JNK is a MAP kinase involved in cellular differentiation, stress response, inflammation, apoptosis and proliferation. Variety of stress such as ultraviolet (UV) radiation a protein synthesis inhibitors, may result in activation of JNK which subsequently phosphorylates c-Jun, a part of the activator protein-1 (AP-1) transcription factor. This activation results in induction of AP-1-dependent target genes involved in cell proliferation and cell death[32].

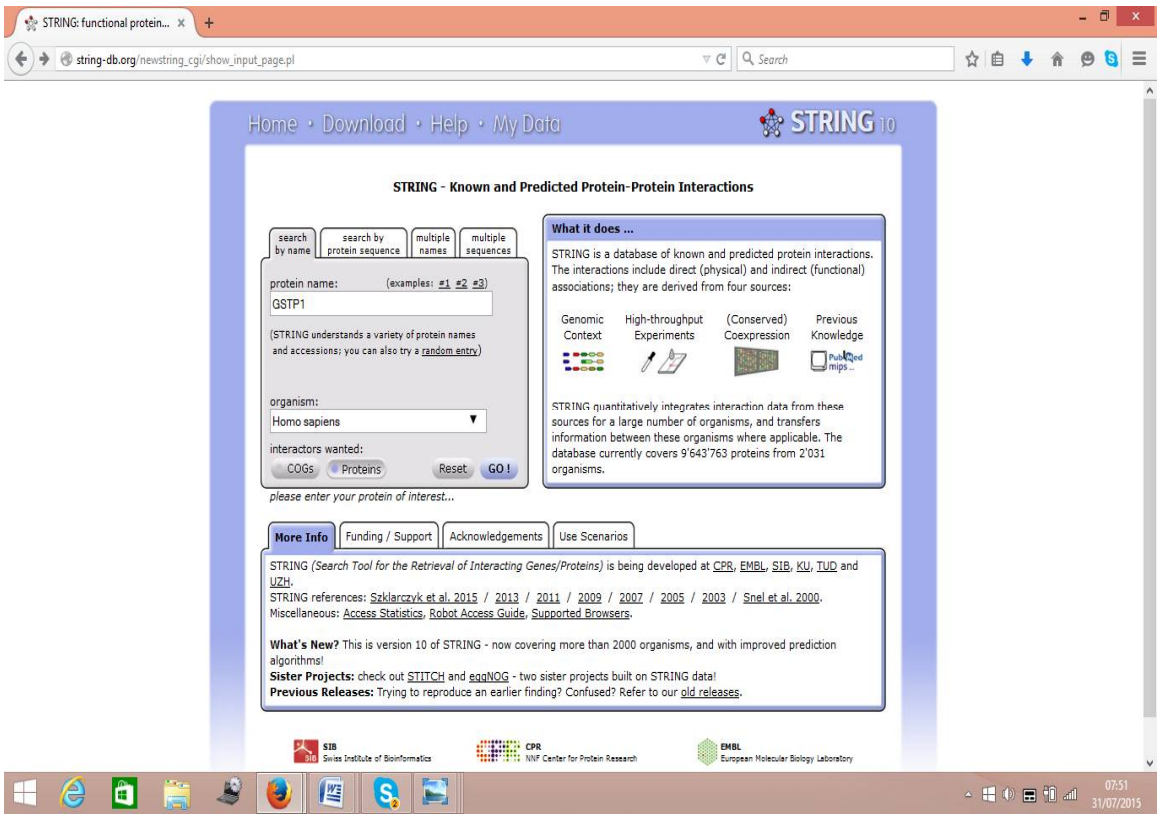


Figure-4
String database interface for finding known interactions and co-expressed genes

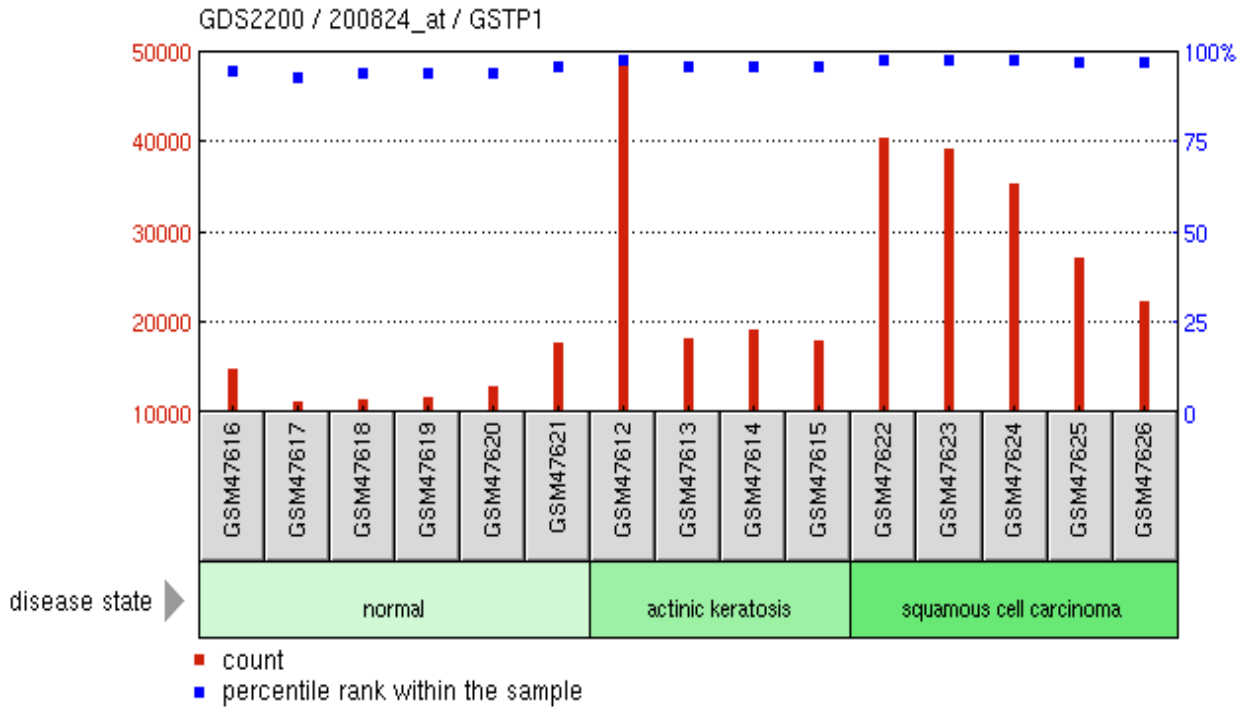


Figure-5
Expression profile of GSTP1 from normal (non-sun-exposed skin), normal (sun-exposed skin), AKs, and SCCs

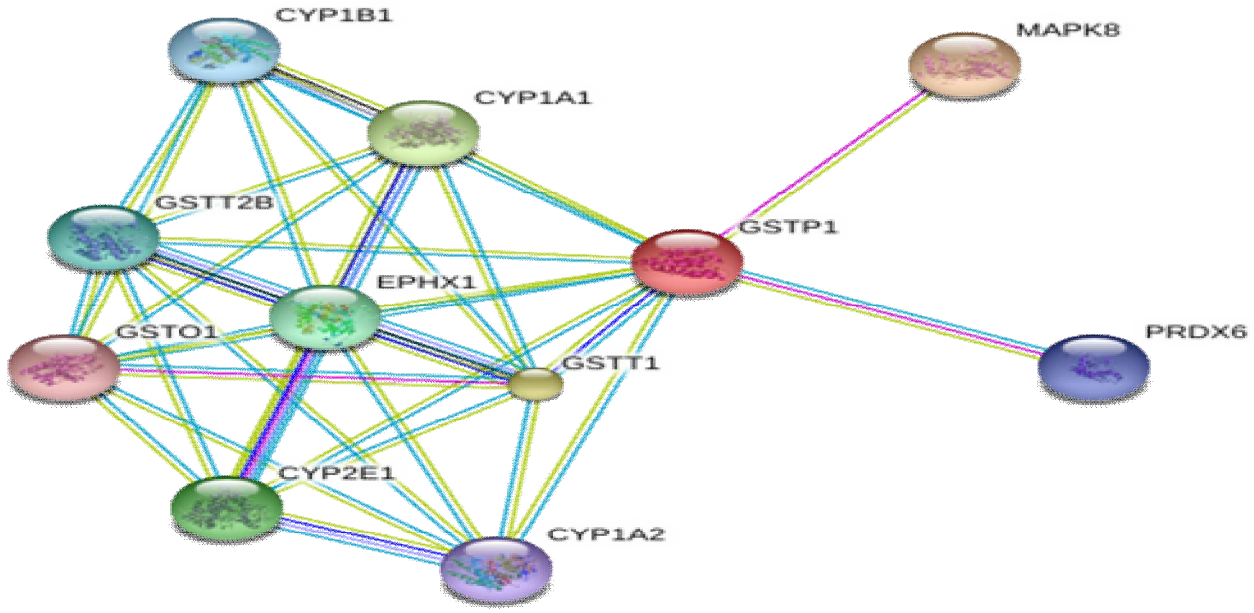


Figure-6
Evidence view of association of GSTP1 with other genes, Different line colors represent the types of evidence for the association

In this research, we have predicted that GSTP1 interact with about 10 different enzymes or proteins (table-1). Each of these enzymes may play significant role in detoxification. Understanding these interactions between GSTP1 and the predicted partners will help in understanding synergistic mechanism of the drug resistance to chemotherapeutics agent by the cancer cells. The score of these interactions were given in table 1 and it indicates the degree of the association. Mitogen-activated protein kinase 8 (MAPK8) was found to strongly interact with GSTP1 and it has the highest score of 0.997 with amino acid 427. The least of the interaction was found between Glutathione S-transferase omega 1 (GSTO1) and GSTP1 and the score 0.956 with about 241 amino acids.

A more complex association have shown that GSTP1 interact indirectly with a number of genes (MAP2K4, MAP3K1, CDC42, FASLG, MAPK81P3, NFATC2, HRAS, RHOA, SP1, APP, FOXO3, MAP2K7, JUN, FOS, STAT3, FOXO1, MAPT, MAPKB1P2, CRK, MAPK8, BCL2, BCL2L1, BCL2L1, DUSP16, DUSP4, PXN, IRS1, JUNB, IRS2, DUSP2, DUSP8, ATF2, DUSP1, JUND, DUSP10, and BAD (figure 7). Clear understanding of interactions of these genes with GSTP1 will help in understanding cell signaling involved in expression of GST pi that were earlier reported in cancers of the breast, bladder, pancreas, colon, stomach, lung, neck and head, cervix, ovary, and, as well as soft tissue sarcoma, testicular embryonal carcinoma, glioma and meningioma[7-16]. Accordingly inhibition of GSTP1 would increase the sensitivity of cancer cells to chemotherapeutic agents.

Table-1
Predicted partners of GSTP1

Enzyme/Protein	No. of amino acids	Score
Mitogen-activated protein kinase 8 (MAPK8)	427	0.997
Glutathione S-transferase theta 1(GSTT1)	240	0.993
Cytochrome P450, family 1, subfamily A, polypeptide 1(CYP1A1)	512	0.980
Cytochrome P450, family 2, subfamily E, polypeptide 1(CYP2E1)	455	0.971
Epoxide hydrolase 1, microsomal (EPHX1)	244	0.979
Glutathione S-transferase theta 2B (GSTT2B)	244	0.970
Cytochrome P450, family 1, subfamily B, polypeptide 1 (CYP1B1)	543	0.968
Peroxiredoxin 6 (PRXD6)	224	0.967
Cytochrome P450, family 1, subfamily A, polypeptide 2 (CPY1A2)	516	0.962
Glutathione S-transferase omega 1 (GSTO1)	241	0.956

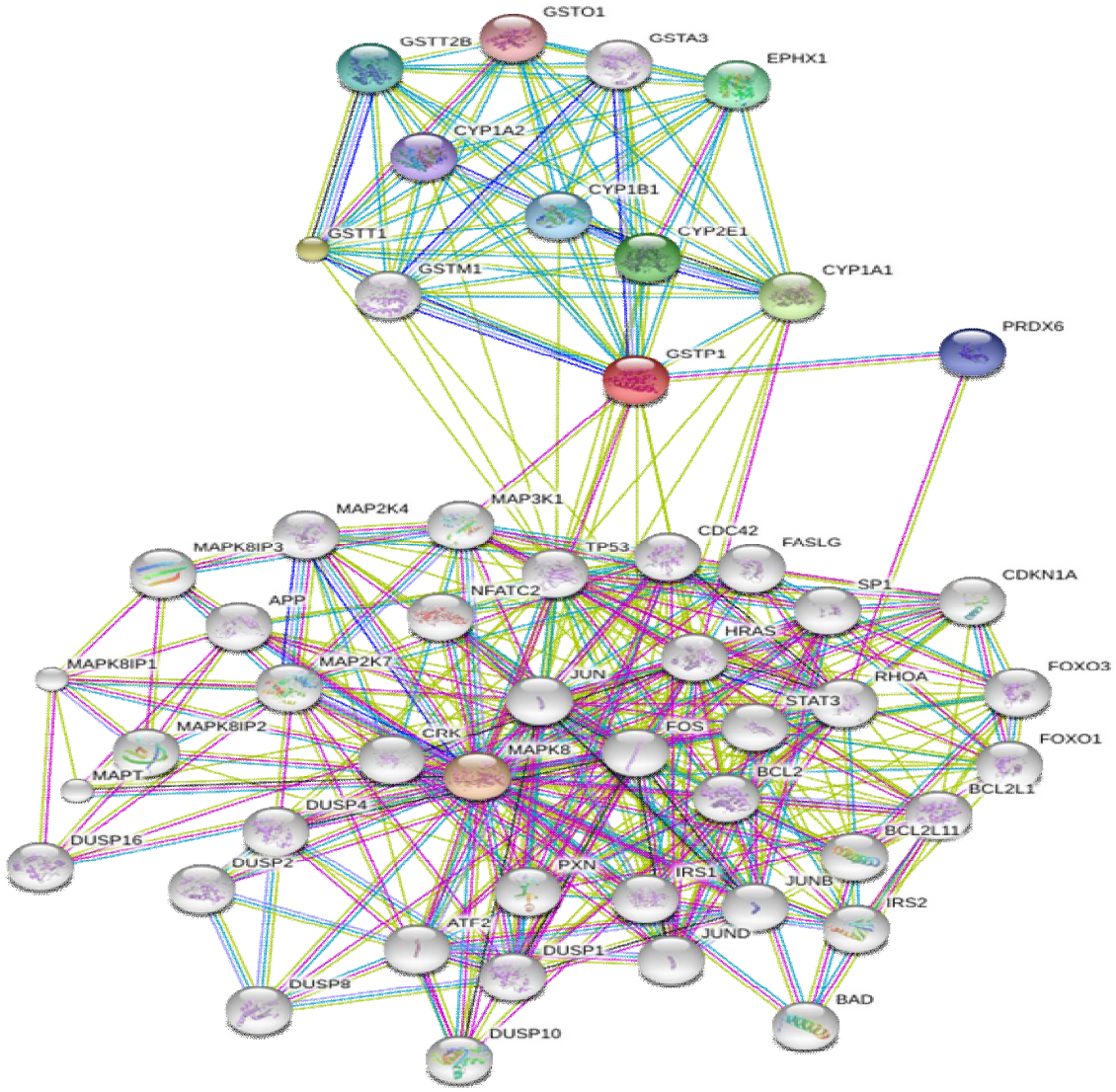


Figure-7
A Complex Evidence view of association of GSTP1 with other genes, Different line colors represent the types of evidence for the association

Conclusion

From the result of the analysis of glutathione s-transferase expression in non melanoma skin cancer using bioinformatics tools, we have arrived at the conclusion that GSTP1 was over-expressed in non melanoma skin cancer. Accordingly inhibition of GSTP1 would increase the sensitivity of cancer cells to chemotherapeutic agents.

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