Systems-Level Analysis of Clinically Different Phenotypes of Juvenile Nasopharyngeal Angiofibromas

Suvi Renkonen, MD; Matti Kankainen, MSc; Jaana Hagström, MD; Caj Haglund, MD; Outi Monni, PhD; Antti A. Mäkitie, MD

**Objectives/Hypothesis:** To explore the molecular genetic background of juvenile nasopharyngeal angiofibromas and to identify biological processes and putative factors determining the different growth patterns of these tumors.

**Study Design:** By comparing copy number and gene expression level changes of two clinically different phenotypes of juvenile nasopharyngeal angiofibromas, we aimed to find processes essential in the growth and development of these tumors. Based on the results and prior knowledge of the proteins significance for growth, we studied the expression of tyrosine kinase SYK in 27 tumor samples.

**Methods:** Comparative genomic hybridization and gene expression analyses were performed for the two tumor samples, and protein expression of SYK was studied in 27 samples by immunohistochemical staining.

**Results:** Between low- and high-stage juvenile nasopharyngeal angiofibromas, 1,245 genes showed at least a two-fold change in expression. The corresponding proteins of these transcripts were enriched in different biological processes. Protein kinase SYK was expressed in all 27 samples, and its intensity significantly correlated with tumor stage.

**Conclusions:** Because the molecular genetic background of juvenile nasopharyngeal angiofibroma is unknown, our aim was to investigate genomic alterations that could associate to low- and high-stage tumors. We were able to identify gene expression changes that relate to particular biological processes, but assessing clinically relevant molecular profiles still requires further characterization. Due to the low incidence of juvenile angiofibroma, in the future a combination of molecular profiling data from several studies would be useful in understanding the molecular background of the disease.

**Key Words:** Nasopharynx, molecular biology, head and neck surgery.

**INTRODUCTION**

Juvenile nasopharyngeal angiofibroma (JNA) is a benign, highly vascular tumor affecting mainly adolescent males. JNA is considered to be the most common benign neoplasm in the nasopharynx, composing 0.5% of all head and neck tumors.1–4 The biomolecular background for the initiation and development of JNA is unsolved. It has been suggested that it is not a true neoplasm, but rather a vascular malformation that occurs due to an uncontrolled process of wound healing and granulation.5,6 JNAs' growth pattern can be locally destructive and cause bone remodeling, enabling life-threatening complications such as intracranial extension. Surgery is the treatment of choice for primary JNA and its recurrences, which typically occur within 1 year of surgery. JNA possesses significant variation among tumors regarding their tendency to recur after surgical removal.7–11

Gene copy number change is one of the mechanisms for a neoplastic cell to control the expression of genes essential to its survival and tumor progression.12–16 The copy number alterations usually involve a large group of genes, located closely in the same chromosome. Only a minority of the genes, located on an abnormally presented chromosomal region that has been associated with a particular cancer model, are typically involved in malignant growth of the tumor.17,18 Other genes may be amplified merely because of their chromosomal proximity with the oncogenic drivers. One way to identify the oncogenic drivers from passengers would be to integrate genome-wide copy number data with gene expression data.19

Due to heterogeneity and low frequency of JNA, its molecular genetic background is poorly known. So far, few studies exist where copy number and gene expression changes in JNA have been reported in a genome-wide manner. Schick et al. were the first to report evidence of genetic imbalance in JNA, based on their comparative genomic hybridization (CGH) study of three patients.20 Since then, several studies have reported

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CGH analyses revealing chromosomal alterations in JNA, in particular gains at chromosomal regions 4q, 6q, 8q, 12, and X, and losses at 17, 22q, and Y. Silveira et al. studied the chromosomal alterations of stromal and endothelial cells separately and found that most imbalances were detected in both components. Despite the numerous chromosomal alterations demonstrated in JNAs, however, the explicit target genes for gains and deletions are still unknown.

Identifying the processes and possible factors determining the growth pattern of JNAs would help us to understand the nature of this tumor and to further develop novel treatment strategies. To explore the molecular genetic background of JNA, and more precisely, the processes putatively determining the different outcomes of JNA tumors, we performed comparative genomic hybridization and gene expression analyses for two clinically different phenotypes of JNA: an endoscopically removed and clearly limited tumor with a diameter of 3 cm and an extensive tumor infiltrating intracranial space and warranting several surgical interventions, radiation treatment, and antiangiogenic therapy. Both tumors were manifested in 15-year-old male patients. Based on these results, we performed immunohistochemical staining of 27 JNA tumor samples. We combined the clinical information with genome-wide copy number as well as gene and protein expression levels to find biological processes and molecular factors that likely explain different growth patterns among these JNA tumors.

MATERIALS AND METHODS

Patients

The fresh JNA tissue samples were prospectively collected from two patients who underwent surgery at our institution between 2008 and 2011. The first patient (high stage [HS]) was 15 years old at the time of diagnosis and had a tumor extending to the pterygopalatinal fossa, sphenoid sinus, ethmoid sinuses, maxillary sinus, orbit, and middle cranial fossa, representing stage IIIa. After the first operation he had recurrent disease and two re-operations. In addition, radiotherapy and antiangiogenic therapy were administered because of persistent intracranial growth. The other patient (low stage [LS]) had a small tumor (3 cm in diameter), limited only to the nasopharynx, and after endoscopic removal has had no evidence of disease. We sampled and snap-froze these JNA tumors in liquid nitrogen within 20 minutes of devascularization and stored them at –80°C to be used for microarray analysis.

Retrospective data of all 27 patients diagnosed with a histologically verified JNA between 1970 and 2008 at the Helsinki University Central Hospital, Helsinki, Finland have been described in our earlier work.

Sample Preparation for Expression and Comparative Genomic Hybridization Microarrays

Total RNA from JNA tissue samples was extracted using Qiagen RNaseasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The integrity of RNA was measured using Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). Two hundred fifty nanograms of total RNA were used for labeling and 12.5 µg of fragmented aRNA were hybridized on microarrays according to the manufacturer’s instructions (Affymetrix, Santa Clara, CA). For DNA copy number analysis, DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen). Two micrograms of genomic DNA were used for array hybridizations according to the manufacturer’s instructions (Agilent Technologies).

Expression Data Analysis

Gene expression profiles of JNA tissue samples were studied using Affymetrix GeneChip Human Genome U133 Plus 2.0 (Affymetrix) gene expression arrays. GeneChip signal intensity data were processed with robust multiarray analysis using remapped gene annotations from the Brainarray Custom CDF files (HG1U33Plus2_Hs_ENSG, v.14.1, http://brainarray.ncbi.nlm.nih.gov). In contrast to the ordinary annotation files with 54,675 probe sets, this custom chip description file has 18,982 probe sets, corresponding to the newest set of Ensembl genes. The significance of differential expression was assessed using the empirical Bayes moderated paired t statistics followed by P value adjustment with the Storey’s approach. Arrays were quality weighted before statistical testing. Genes with P values <0.05 were considered as significantly differentially expressed. All methods used are implemented in the simpleaffy, limma, and qvalue packages of the Bioconductor project.

Comparative Genomic Hybridization Analysis

Copy number variations in JNA tissue samples were studied using Agilent Human Genome CGH 244A Oligo Microarray (Agilent Technologies). Array intensity data were processed with Agilent Feature Extraction tool (Agilent Technologies). Probes associated with genomic coordinates according to manufacturer’s annotation were selected and coordinated (NCBI36/hg18) converted to the newest genome build (GRCh37/hg19) using LiftOver. For copy number variation (CNV) status of genes, averages were taken over probes located within the start and end coordinate of Ensembl gene models. Similar to gene expression data, CNV status is given as log2 transformed values. Gender-matched DNA obtained from white blood cells of healthy individuals (Promega Corp., Madison, WI) was used as a reference for CGH array hybridizations.

Gene Enrichment Set Analysis

The gene ontology categories provide a controlled vocabulary to describe the gene and the gene product attributes of any organism. The gene ontology (GO) rilla Gene Enrichment Analysis and Visualization Tool was used to discover gene ontology terms enriched at either end of a gene list sorted according to the moderated t test score calculated using limma. All three gene ontology categories were tested. Functional groups enriched among statistically significantly differentially expressed genes were tested with DAVID (Database for Annotation, Visualization, and Integrated Discovery).

Protein-Protein Interaction Networks

Web-based protein interaction network analysis platform (PINA) provided integrated protein-protein interaction data from six databases. This tool was exploited to identify the interactor proteins of investigated proteins.

Immunohistochemistry

For immunohistochemistry, we used formalin-fixed and paraffin-embedded samples of JNA and mouse monoclonal
antibody for SYK (1:1000, clone 4D10.1; abcam, Cambridge, UK) as described earlier. 40 For vessel density, vessels marked by mouse monoclonal CD31 (1:20, clone JC70A; Dako, Glostrup, Denmark) were counted, and the mean counts per millimeter squared were calculated.

Statistical Analysis
SPSS version 15.0 software (SPSS, Inc., Chicago, IL) was used for statistical analyses. Categorical variables were cross-tabulated and analyzed using the Fisher exact test or Spearman correlation. All P values are two-sided, and P values ≤ 0.05 were considered significant.

RESULTS
Gene Copy Number Aberrations
The gene copy numbers from both the LS and HS specimens were compared to normal male control specimens. The copy number ratios (gains and losses) were then compared between the two samples. The log2 cutoff values for gains and losses were 1 and −1, respectively, indicating a two-fold difference in copy number. Forty-six genes showed at least a two-fold copy number difference between the LS and HS samples and their corresponding controls (Table I). Eleven genes were altered in LS but not in HS, and 20 genes were altered in HS but not in LS, when compared to the control. When compared with each other, the copy number change between LS and HS was at least two-fold in 11 genes (Fig. 1).

Gene Expression Levels
At least a two-fold change in the gene expression levels between LS and HS was seen in 1,383 transcripts, of which in 1,245 cases the q value was <0.05 (Supplementary Table I). In 773 transcripts the level was higher in LS, and in 610 the level was higher in HS. Copy number-related change of gene expression levels was seen in three genes (Fig. 1).

Gene Ontology
We then performed an enrichment analysis aimed at detecting categories enriched among genes with altered expression status. 41 Assessment of categories with GOrilla revealed some strongly enriched categories, as shown in Tables II and III.

One of the genes that had over a two-fold change in expression between LS and HS was tyrosine kinase SYK, which we decided to further study computationally as well as in terms of immunohistochemical analysis. To identify proteins interacting with SYK, we used the PINA protein interaction database tool. 39 We found 95 proteins interacting with SYK, and in 12 of these the gene expression change between LS and HS was over two-fold (Supplementary Table II, Fig. 2). These genes, together with SYK, were more frequently present in the GO-categories enriched in HS, when compared to those enriched in LS (Table III).

Histology of LS and HS Samples
Vessel densities of the two samples were as follows: in LS 290 vessels per millimeter squared, and in HS 174 vessels per millimeter squared. In LS there were more slit-like vessels than large vessels, and in HS there were almost exclusively slit-like vessels. Both samples had high cellularity.

Protein Expression of SYK
Cytoplasmic SYK protein expression in stromal cells (Fig. 3) was detected in all JNA tissue samples analyzed (27/27, 100%). The frequency of SYK expression levels varied from very low to high, being very low in seven, low in 10, moderate in seven, and high in two samples (Fig. 4). SYK was expressed in LS and HS and was evaluated to be low in both samples.

Correlations Among Histology, Immunohistochemistry, and Clinical Parameters
We studied correlations among protein expression levels of SYK with clinical parameters and found a significant correlation with lower tumor stage (Fisher exact, P = 0.048). We found no other correlations.

DISCUSSION
We compared two different phenotypes of JNA i.e., an HS tumor with persistent disease despite several therapeutic interventions and an LS tumor managed with a single endoscopic surgery with no recurrences. This study was carried out to clarify the diverse clinical behavior between large tumors with progressive growth and LS tumors with no progression. By combining the data of copy number (DNA), gene expression (mRNA), and protein expression levels with clinical outcome of our 27 JNA patients, we aimed to characterize the factors and processes leading to different tumor growth patterns. Analyzing the data of a LS and HS JNA tumor, we found a few copy number changes that link to changes in the gene expression and 1,245 genes that had a significant, over two-fold change in their expression between LS and HS JNA tumors. The in silico-translated proteins of differently expressed transcripts showed enrichment for different biological processes and functions in the phenotypically different tumors, and the enriched categories are in concordance with earlier reports of protein expression in JNA. By immunohistochemistry, we analyzed the protein expression of protein kinase SYK, as one example of a protein with altered gene expression status, and found that SYK expression correlated with lower tumor stage. This indicates the idea of SYK possibly being centered in JNA pathogenesis, which has already been shown in various other tumors.

The low level of copy number changes might be due to the heterogeneity of the tumor consisting of epithelial, stromal, and endothelial cells. As the neoplastic cell in JNA is unknown, the putative heterogeneity of the tumor samples may affect the detection of chromosomal abnormalities. 26 Another possible explanation is that in
### TABLE I.

Copy Number Ratios in Low- and High-Stage Juvenile Nasopharyngeal Angiofibromas When Compared to Normal Male Controls.

<table>
<thead>
<tr>
<th>Ensembl Gene ID</th>
<th>External Gene ID</th>
<th>Description</th>
<th>Chr</th>
<th>LS</th>
<th>HS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENSG00000178403</td>
<td>NEUROG2</td>
<td>Neurogenin 2</td>
<td>4q25</td>
<td>1.31*</td>
<td>2.27*</td>
</tr>
<tr>
<td>ENSG00000167034</td>
<td>NKX3-1</td>
<td>NK3 homeobox 1</td>
<td>8p</td>
<td>1.57*</td>
<td>1.89*</td>
</tr>
<tr>
<td>ENSG00000187642</td>
<td>C1orf170</td>
<td>Chromosome 1 open reading frame 170</td>
<td>1p36.33</td>
<td>1.52*</td>
<td>1.80*</td>
</tr>
<tr>
<td>ENSG00000182424</td>
<td>C11orf72</td>
<td>Chromosome 11 open reading frame 72</td>
<td>11q13.2</td>
<td>1.34*</td>
<td>1.77*</td>
</tr>
<tr>
<td>ENSG00000121551</td>
<td>PHF1</td>
<td>PHD finger protein 1</td>
<td>6p21.32</td>
<td>1.12*</td>
<td>1.64*</td>
</tr>
<tr>
<td>ENSG00000184441</td>
<td>AP001062.7</td>
<td></td>
<td>21g22.3</td>
<td>0.87</td>
<td>1.61*</td>
</tr>
<tr>
<td>ENSG00000235608</td>
<td>NKX1-1</td>
<td>NK1 homeobox 1</td>
<td>4p16.3</td>
<td>1.32*</td>
<td>1.55*</td>
</tr>
<tr>
<td>ENSG00000181865</td>
<td>NEUROG1</td>
<td>Neurogenin 1</td>
<td>5q31.1</td>
<td>1.33*</td>
<td>1.40*</td>
</tr>
<tr>
<td>ENSG00000108551</td>
<td>RASD1</td>
<td>RAS, dexamethasone-induced 1</td>
<td>17p13.33</td>
<td>1.14*</td>
<td>1.37*</td>
</tr>
<tr>
<td>ENSG00000184441</td>
<td>AP001062.7</td>
<td></td>
<td>21g22.3</td>
<td>0.87</td>
<td>1.61*</td>
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<td>NKX1-1</td>
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<td>4p16.3</td>
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<td>ENSG00000181865</td>
<td>NEUROG1</td>
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<td>1.33*</td>
<td>1.40*</td>
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<td>RASD1</td>
<td>RAS, dexamethasone-induced 1</td>
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<td>AP001062.7</td>
<td></td>
<td>21g22.3</td>
<td>0.87</td>
<td>1.61*</td>
</tr>
</tbody>
</table>

The log2 cutoff values for gains and losses were 1 and 1, respectively, indicating a two-fold difference in copy number.

*Genes with over a two-fold change.

ChROMosome; LS = low-stage tumor; HS = high-stage tumor; PHD = plant homeo domain, RAS = rat sarcoma, THAP = thanatos associated protein, SRY = sex determining region Y, GPR = G-protein coupled receptor, BTB = BR-C, ttk, bab (Broad-complex, tramtrack, bric a brac), POZ = Pox virus and Zinc finger, KISS = kisspeptin, SREBF = sterol regulatory element binding transcription factor, HLA = human leukocyte antigen, MHC = major histocompatibility complex.
these subjects the central level of regulation is not due to copy number abnormalities.

The comparison of gene expression levels revealed statistically significant changes in 1,245 genes. Genes overexpressed in the LS tumor with no recurrences were often associated with biological processes and functions linked to the organization of the tumor, such as development of the vasculature and epithelium, cell adhesion and cell-cell junctions, collagen catabolic processes, and hormone activity, whereas genes upregulated in the HS tumor with recurrences were associated with, for example, signal transduction activity (including positive regulation of phosphorylation), tumor necrosis factor production, and Wnt-activated receptor activity (Tables II and III).

Our observations are comparative with former reductionistic data of JNA in many cases. Observations of the expression of proangiogenic factors in benign but strongly vascularized JNA have been shown first by Schiff, who described the expression of basic fibroblast growth factor in the endothelium of JNA, and later by other studies presenting the expression of transforming growth factor and the most prominent proangiogenic growth factor, vascular endothelial growth factor, as well as its receptors. The expression of hypoxia inducible factor has been shown in both stromal and endothelial cells of JNA, although its possible correlation with vessel density remains controversial. Also, it has been shown that JNA is a collagen-rich tumor, where the

**TABLE II.** Low-Stage Angiofibroma: The Enriched Gene Ontology Categories of Genes With Over a Two-Fold Difference Between the Low- and High-Stage Juvenile Nasopharyngeal Angiofibromas.

<table>
<thead>
<tr>
<th>GO-Term</th>
<th>Description</th>
<th>P Value</th>
<th>Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0008544</td>
<td>Epidermis development</td>
<td>1.7E-14</td>
<td>8.46</td>
</tr>
<tr>
<td>GO:0009888</td>
<td>Tissue development</td>
<td>4.02E-14</td>
<td>3.78</td>
</tr>
<tr>
<td>GO:0001525</td>
<td>Angiogenesis</td>
<td>6.1E-13</td>
<td>3.65</td>
</tr>
<tr>
<td>GO:0030334</td>
<td>Regulation of cell migration</td>
<td>1.77E-12</td>
<td>2.44</td>
</tr>
<tr>
<td>GO:0007155</td>
<td>Cell adhesion</td>
<td>4.77E-12</td>
<td>2.10</td>
</tr>
<tr>
<td>GO:0009611</td>
<td>Response to wounding</td>
<td>9.6E-11</td>
<td>2.31</td>
</tr>
<tr>
<td>GO:0001666</td>
<td>Response to hypoxia</td>
<td>1.25E-8</td>
<td>2.91</td>
</tr>
<tr>
<td>GO:0008284</td>
<td>Positive regulation of cell proliferation</td>
<td>1.31E-8</td>
<td>1.35</td>
</tr>
<tr>
<td>GO:0043535</td>
<td>Regulation of VEGFR-signaling</td>
<td>1.34E-6</td>
<td>8.63</td>
</tr>
<tr>
<td>GO:0030574</td>
<td>Collagen catabolic process</td>
<td>1.99E-4</td>
<td>12.96</td>
</tr>
</tbody>
</table>

The genes upregulated in the low-stage angiofibroma were enriched in several gene ontology (GO) classes, of which selected enrichments are displayed.

Enrichment is defined as follows: N is the total number of genes; B is the total number of genes associated with specific null; n is the number of genes in the top of the user’s input list or in the target set when appropriate; b is the number of genes in the intersection; enrichment = (b/n)/(B/N).

VEGFR = vascular endothelial growth factor receptor.

**TABLE III.** High-Stage Angiofibroma: The Enriched Gene Ontology Categories of Genes With Over a Two-Fold Difference Between the Low- and High-Stage Juvenile Nasopharyngeal Angiofibromas.

<table>
<thead>
<tr>
<th>GO-Term</th>
<th>Description</th>
<th>P Value</th>
<th>Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0002682</td>
<td>Regulation of immune system process</td>
<td>7.9E-16</td>
<td>2.19 (13/133)</td>
</tr>
<tr>
<td>GO:0016477</td>
<td>Cell migration</td>
<td>2.81E-7</td>
<td>1.90 (7/76)</td>
</tr>
<tr>
<td>GO:0019932</td>
<td>Second messenger-mediated signaling</td>
<td>5.82E-5</td>
<td>7.54</td>
</tr>
<tr>
<td>GO:0043066</td>
<td>Negative regulation of apoptotic process</td>
<td>6.52E-5</td>
<td>1.93 (2/32)</td>
</tr>
<tr>
<td>GO:0043069</td>
<td>Negative regulation of programmed cell death</td>
<td>8.57E-5</td>
<td>1.91 (2/49)</td>
</tr>
<tr>
<td>GO:0032760</td>
<td>Positive regulation of tumor necrosis factor production</td>
<td>5.35E-4</td>
<td>10.82 (2/17)</td>
</tr>
<tr>
<td>GO:0042813</td>
<td>Wnt-activated receptor activity</td>
<td>8.09E-4</td>
<td>28.62</td>
</tr>
</tbody>
</table>

The genes upregulated in the high-stage angiofibroma were enriched in several gene ontology (GO) classes, of which selected enrichments are displayed. When SYK or any of its 95 interacting proteins were among the enriched genes, their proportion of all genes is shown in parentheses.

Enrichment is defined as follows: N is the total number of genes; B is the total number of genes associated with specific null; n is the number of genes in the top of user’s input list or in the target set when appropriate; b is the number of genes in the intersection; enrichment = (b/n)/(B/N).
specific extracellular matrix degradation properties are essential for enabling tumor growth and remodeling.\textsuperscript{47,48} Duerr et al. described significant expression of matrix metalloproteinases with gelatinase/collagenase activity in JNA and suggested that these might be involved in the growth and angiogenesis on JNA.\textsuperscript{47}

Several reports have revealed an association between JNA and familial adenomatous polyposis (FAP), JNAs being 25 times more frequent in patients with FAP than in the normal age-matched population.\textsuperscript{49–51} FAP is known to have extracolonic manifestations, such as desmoid tumors and benign epidermoid cysts. Germ-line mutations of the APC gene are known to be systemically present in FAP.\textsuperscript{51} One known function of the APC gene product is the regulation of B-catenin, a transcriptional activator of Wnt signaling pathway and a component of the cadherin-mediated cell-cell adhesion mechanism.\textsuperscript{52} In 2011, Silveira et al. showed a correlation between gene copy number and gene expression levels in ASPM, CDH1, CTNNB1, FGF18 and SUPT16H, proteins that are known to participate in Wnt/B-catenin pathway. Another finding was the overexpression of AURKB1 in JNA, which they complemented with AURKB-targeted molecular cancer therapies.\textsuperscript{28} Interestingly, in our study, we found that proteins linked to tumor necrosis factor (TNF) production were upregulated in the HS JNA tumor. TNF-$\alpha$–targeted molecular therapies are in wide use in cancer and immunological diseases, and thus this finding is worth further investigations as a possible new target for molecular therapy.\textsuperscript{53,54}

At least 518 protein kinases are encoded by the human genome. Interest in the investigation of protein kinases is partly explained by the successful development of protein kinase inhibitors, largely for oncological targets. Spleen tyrosine kinase SYK is a nonreceptor tyrosine kinase, originally cloned from porcine spleen. Activated SYK phosphorylates its substrates, including other enzymes and adaptor proteins, conducting various cellular responses, such as cell proliferation, differentiation, and survival.\textsuperscript{55,56} SYK has been linked to the development of various malignancies and is required for the oncogenic activity of several viruses, such as Epstein-Barr virus.\textsuperscript{57} In breast cancer, SYK acts as a tumor suppressor. It is present in normal breast tissue, benign lesions, and low-tumorigenic cell lines, but in invasive cancer, SYK mRNA and protein levels are low or undetectable. Transfection of SYK in these tumors suppresses tumor growth and metastasis formation.\textsuperscript{58,59}
Curiously, in chronic leukemia, gastric cancer, and oral squamous cell cancer, SYK acts as an oncogene, as high SYK expression correlates with tumor progression and has prognostic value. In this study, we showed that phosphorylation processes were enriched in the upregulated genes of HS JNA tumor. We also found SYK gene expression to be significantly higher in the HS JNA tumor, and found a reverse correlation between SYK expression and tumor stage. The presence of SYK protein is mostly reflected by the expression of its mRNA, indicating that its regulation takes place at the transcriptional level. Additionally, epigenetic gene silencing has been shown to be causally responsible for SYK loss of expression. However, the opposite effects of SYK in different tumors still require further investigation. Alternative splicing of SYK has been shown to be crucial in the development of breast cancer.

**CONCLUSION**

Identification of clinically relevant genetic alterations is important to assess the molecular genetic background of JNA. Because the incidence of JNA is very low, all of the molecular data published regarding the disease will be valuable in understanding JNAs molecular genetic background. The application of discovery-driven methodologies on diseases with unknown etiologies can provide new hypotheses worth further analyses. Learning novel, unforeseen aspects from molecular mechanisms of complex, multifactorial diseases like JNA can provide future targets for individual treatment strategies.

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**BIBLIOGRAPHY**


