Molecular biology of juvenile nasopharyngeal angiofibroma

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Abstract

Juvenile nasopharyngeal angiofibroma (JNA) is a rare benign tumour, mostly affecting adolescent males. Its molecular aetiology and pathogenesis are widely unknown. In the last two decades several different genes and proteins have been analyzed by various molecular approaches to identify possible mechanisms contributing to JNA growth. In this review we give a short overview about the published basic JNA research and suggest future directions.

Key words: *juvenile nasopharyngeal angiofibroma, genetics, comparative genomic hybridization, cellular changes.*

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Current concepts

Juvenile nasopharyngeal angiofibroma (JNA) is a rare histologically benign tumour, which arises generally from the postereolateral wall of the nasal cavity. The tumour is characterized by its strong vascularisation [1]. Despite its benign character, an aggressive growth pattern with intracranial tumour extensions is frequent [2, 3]. The pathogenesis of JNA is still unclear. However, two mechanisms might be suggested by the clinical picture of the disease. First, a causal association between JNA and familial adenomatous polyposis (FAP) genesis has been proposed, because JNAs occur 25 times more frequently in patients with FAP compared to age-matched groups [4, 5]. Second, because JNA mainly affects male adolescent boys, a role for androgens in JNA growth has been suggested [6, 7].

FAP and JNA

FAP results from mutations in the adenomatous polyposis coli (APC) gene located on chromosomal arm 5q. The APC gene product is part of an activation complex regulating the cytoplasmic level of β-catenin. Dysregulation of β-catenin results in the activation of the Wnt pathway and subsequently the genesis of FAP. Analyzing the APC/β-catenin pathway in JNA, mutations of the APC gene were not found with the exception of a single frameshift mutation [5, 8-10]. However, β-catenin mutations were found in 12 out of 16 analyzed JNAs [9]. Additionally, high nuclear β-catenin expression levels in stromal cells of JNA [9, 11] as well as in epithelial cells [12] were detected by immunohistochemical analyses indicating transcriptional activation. Thus, there is evidence that alterations of the β-catenin gene might be involved in JNA tumorigenesis.

JNA and steroids

Increased androgen receptor (AR) staining was identified by immunohistochemistry in 18 out of 24 analyzed cases [6]. This corresponds to the reported duplication of the AR gene in five out of seven JNAs reported by Schick et al. [7]. In another study neither oestrogen receptors, progesterone receptors nor increased AR were identified [13]. Accordingly, in a study reported by Shikani and Richtsmeier [14] androgens failed to stimulate JNA growth in nude mice and in vitro. Taken together, these data are conflicting and suggest that steroid receptor dysregulation is not the only cause of JNA genesis.

Growth factors and receptors

In order to identify a possible role of growth factors in JNA tumorigenesis, various cytokines have been analyzed. Nagai et al. [15] investigated 20 JNA for the

expression of several cytokines at the mRNA-level. He found basic fibroblast growth factor (bFGF) overexpressed in two out of 17 cases, platelet-derived growth factor A (PDGF-A) in one out of 12 cases, platelet-derived growth factor B (PDGF-B) in four out of eight cases, insulin-like growth factor II (IGF-II) in nine out of 17 cases, transforming growth factor beta 1 (TGF-β_1) in one out of 18 cases, and vascular epithelial growth factor (VEGF) in four out of 20 cases. Confirming results have been reported by several other groups: Schiff et al. [16] detected bFGF, a high expression level of this cytokine was reported by Zhang et al. [11] and Schuon et al. [17], overexpression of IGF-II was described by Coutinho-Camillo et al. [18], and the upregulation of TGF-β_1 by Saylam et al. [19], Schuon et al. [17] and of activated TGF-β_1 by Dillard et al. [20]. Finally, VEGF and its main receptor FLK-1 have been identified at increased levels [1, 17, 19]. Taken together, these findings suggest that growth factors, especially with angiogenic properties, might be involved in the promotion of JNA growth.

Some of the other genes analyzed

Several proto-oncogenes and oncogenes have been analyzed. No mutations were identified, e.g. in the Ha-ras and Ki-ras oncogenes [21] mRNA overexpression of c-fos was detected in three out of 24 analyzed tumours [15]. A high expression level of the c-kit gene product was identified using an immunohistochemical approach in 12 JNAs [11]. FISH analyses of the proto-oncogene c-myc revealed heterogeneous results with losses of the gene at lower stages of the disease and gains at advanced stages [22]. Analyzing Her-2/neu by the same method, gene losses were identified in five out of seven cases and, despite the observed losses, mRNA upregulation was detected by semi-quantitative RT-PCR analysis in two of the cases [23]. The mRNA of the tumour suppressor gene p53 was upregulated in nine out of 22 JNAs [15] and in another study in four out of seven JNAs [23]. Taken together, the analyzed growth promoting genes seem to be stimulated in a portion of JNA. Consequently, proliferating cell nuclear antigen (PCNA), a marker of cellular proliferation, has been detected at high levels in all JNAs analyzed [19].

Whole genome analysis

The comparative genomic hybridization (CGH) analysis technique was introduced by Kallionemi et al. in 1992 [24]. This technique enables the comprehensive analysis of DNA gains and losses in the genome of a given cell.

The CGH technique has been applied for JNA analysis by two groups. We analyzed 22 JNAs including six recurrences and identified autosomal genomic alterations only in six cases [25]. Frequent DNA gains occurred on chromosomal arms 12q and 16p (5x), 1p (4x), 10q, 19q, and 20q (3x). Additionally, gonosomal genomic alterations were found in 15 cases. The group of Schick reported about 29 JNAs [26]. Parts of these analyses have already been published in two papers before [27, 28]. This group identified autosomal genomic alterations in 27 out of 29 cases. Frequent DNA gains occurred on chromosomal arms 4q (12x), 6q (10x), 12q (7x), 13q, and 19p (6x). Frequent DNA losses were found on 8p and 22q (14x), 16p and 17p (11x), 1p and 17q (10x), 5q and 15q (9x), 16q (8x), 9q, 10q, 19q, 20q, and 21q (6x). Common to both studies was the finding of gains on the chromosomal arms 12q and 19p in more than 20% of all analyzed tumours. Gonosomal genomic alterations were identified in 27 JNAs. Both groups reported the frequent loss of genomic material of the Y chromosome and of gains of the X chromosome. The gains on the X chromosome might contribute to the overexpression of c-fos [15] and to the gains of the androgen receptor [7]. However, findings concerning gonosomal alterations should be handled with caution because of the methodical limitations of the CGH technique.

Candidate genes

The two affected loci on autosomes identified frequently by CGH are 12q14-q24 and 19p13.1-13.2. Numerous genes identified by database search (http://www.ncbi.nlm.nih.gov) [29] are generally involved in fundamental processes of cellular signalling, growth and differentiation and might therefore contribute to JNA genesis. Several members of the ras oncogene family, regulators of ras signalling and proto-oncogenes have been detected (Table 1). As we speculated in an earlier paper, members of the superfamily of the ras-like small GTPases might be involved in JNA tumorigenesis [25]. Another good candidate is insulin-like growth factor 1 (IGF1). It was shown recently that IGF1 is overexpressed in younger patients with benign prostatic hyperplasia [30]. Thus, IGF1 might act in concert with other in JNA upregulated growth promoting and angiogenic cytokines in JNA. At chromosomal region 12q22-q23 Gpr49 (synonym: LGR5) is located. Gpr49 belongs to the glycoprotein hormone receptors family. It plays a role in carcinoma development in concert with β-catenin mutations. It is assumed that Gpr49 is upregulated by mutant β-catenin [31]. As β-catenin mutations were frequently identified in JNAs [9], Gpr49 (LGR5) might be part of the dysregulated mechanisms in JNA.

Table 1. Candidate genes located on chromosomal arms 12q and 19p

Code	Localization	Name/classification	Known function/involved in
Analyzed region: 12q14-q24			
DYRK2	12q15	dual-specificity tyrosine-(y)-phosphorylation regulated kinase 2	cellular growth
RAP1B	12q14	member of ras oncogene family	cellular signalling
NUP107	12q15	nucleoporin 107 kDa	nuclear pore complex regulation
FRS2	12q15	fibroblast growth factor receptor substrate 2	cellular signalling
RAB3IP	12q14.3	RAB3a interacting protein	interaction with cancer-related protein SSX2
PTPRB	12q15	protein tyrosine phosphatase, receptor type B	cellular signalling
TSPAN8	12q14.1-21.1	tetraspanin 8	cellular growth, expressed in different carcinomas
LGR5/Gpr49	12q22-q23	leucine-rich repeat-containing G-protein coupled receptor 5	carcinoma development together with β-catenin mutations
RAB21	12q21.1	member of the ras oncogene family	influences traffic of integrin
TBC1D15	12q21.1	TBC1 domain family, member 15	regulation of RAB GTPase activity
NAP1L1	12q21.2	nucleosome assembly protein-1 like 1	cell proliferation
CSRP2	12q21.1	cysteine and glycine rich protein 2	regulation of cell growth
E2F7	12q21.2	E2F transcription factor 7	regulation of cell cycle progression
LOC441643	12q21.31	similar to p53 and DNA damage-regulated protein	cellular growth
NTN4	12q22-q23	netric 4	role in breast cancer
ELK3	12q23	ELK3, ETS-domain protein (SRF accessory protein 2)	activates transcription in the presence of ras
IGF1	12q22-q23	insulin-like growth factor 1 (somatomedin C)	connected with some cancers
PRDM4	12q23-q24.1	PR domain containing 4	cell differentiation
PTPN11	12q24	protein tyrosine phosphatase, non-receptor type 11	regulation of cell signalling events
Analyzed region: 19p13.1-13.2			
GDF1	19p12	growth differentiation factor	regulation of cellular growth
GDF15	19p13.11	growth differentiation factor	tissue differentiation
RAB3A	19p13.2	member of the ras oncogene family	cellular signalling
JAK3	19p13.1	janus kinase 3	cellular signalling
RAB8A	19p13.1	member of the ras oncogene family	cellular signalling
PRKACA	19p13.1	protein kinase, c-AMP-dependent, catalytic	cellular signalling
RFX1	19p13.1	regulatory factor	modulator of ras signalling
BTBD14B	19p13.13	BTB (POZ) domain containing 14 B	cellular growth
JUNB	19p13.2	jun B proto-oncogene	tumour development
CDC37	19p13.2	cell division cycle 37 homologue	control of cell division cycle
RAB11B	19p13.2	member of the ras oncogene family	cellular signalling
ARHGEF18	19p13.3	rho/rac guanine nucleotide exchange factor	cellular signalling

Perspectives

In a recent paper the Schick group proposed the stimulation of the androgen receptor by β-catenin and thereby receptor mediated transcriptional activation as a potential mechanism of JNA growth [12]. Within this concept the observed growth characteristics could be interpreted as pathophysiological consequences of androgen receptor stimulation due to β-catenin dysregulation. This hypothesis would merge the two

concepts of JNA genesis – FAP-associated and androgen-driven. The available results fit in this hypothesis, although the genetic data suggest that additional genes are involved, probably triggering the disease. However, further studies concerning the function of the potentially involved gene products are mandatory. In future, targeting the Wnt-pathway with its key player β-catenin might open new paths of JNA disease management.

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