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Molecular interactions in juvenile nasopharyngeal angiofibroma: preliminary signature and relevant review

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Abstract

Background The molecular profile of juvenile nasopharyngeal angiofibroma (JNA) is extremely variable. In absence of established molecular signature the molecular targeting seems difficult for this heterogeneous disease. To establish a basic molecular signature, this paper analyses the interaction of 7 markers according to their ranks as per the decreasing scale of molecular expression.

Materials and methods Fourteen samples of JNA were obtained following surgical excision and mRNA expressions were established through real-time polymerase chain reaction (RT-PCR) for vasculoendothelial growth factor (VEGF), fibroblastic growth factor (FGF), c-Kit, c-myc, Ras, platelet-derived growth factor (PDGF) and tumor suppressor gene p53. Nasal polyp was taken as control. The quantitative expressions for every marker were ranked on a decreasing scale and were compared by Spearman's rank correlation test to define the statistically significant interaction. An attempt was also made to overview the basic clinical parameters (age, duration of symptoms, radiological staging, intraoperative haemorrhage and tumor-volume/weight) associated with enhanced molecular expressions for every marker. Results: Five significant molecular interactions were identified on the basis of rank-correlation: (1) FGF/VEGF ($p < 0.01$); (2) Ras/FGF ($p < 0.01$); (3) Ras/VEGF ($p < 0.001$), (4) FGF/c-Kit ($p < 0.05$); (5) c-Myc/p53 ($p < 0.05$). These basic 'molecular signatures' suggested a preliminary 'molecular classification'. The implication of the interactions between FGF, VEGF and Ras were the most outstanding observation that not only revealed a direct relationship but were also consistent with the clinical behaviour. In addition, a non-significant interaction was identified with c-Myc/PDGF and also an inverse relationship between FGF/c-Kit.

Conclusions FGF, VEGF, and Ras being significantly interrelated seemed to be the 'most soft' molecular targets for JNA. The other targets observed included FGF/c-Kit and c-Myc/p53 interactions that seemed equally important but only after VEGF/FGF/Ras complex per se. These preliminary signatures are likely to provide a background for further expansion of the molecular classification of JNA.

Keywords Juvenile nasopharyngeal angiofibroma · Molecular interactions · VEGF · FGF · C-Kit · c-Myc · Ras · PDGF · P53

Introduction

Juvenile nasopharyngeal angiofibroma (JNA) is an extremely vascular, locally invasive tumour seen around nasopharynx. A wide variation has been reported with respect to geographical [1, 2], clinical [3] and molecular [2] contexts while its growing incidence in recent era [1] points to the evolving molecular mechanisms [4] including the implication of human papilloma virus (HPV) [5]. Such extreme variability reflects the corresponding underlying differences in its molecular constitution as well. Hence, there is a need for a better understanding of underlying molecular mechanisms/inter-molecular interactions that, however, is lacking due to

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paucity of the literature and limitations in the number of markers (1 or 2) studied at a time. Such a molecular understanding may better define the etiopathogenesis, as well as further suggest the possibility of molecular targeted therapy as an alternative to morbid surgery. Accordingly, the underlying molecular interactions need to be defined and some basic molecular signatures/classification need to be established for this heterogenous disease. More importantly, these molecular signatures need to be linked with specific clinical phenotypes to further predict the molecular surrogate of clinical behaviour. The majority of literature with a single (or 2) marker study does not necessarily unfold the role/interaction of many more markers instrumental therein. Hence this paper aims to study the molecular interactions between 7 established markers in a single cohort of patients, (already described elsewhere [4] along with their influence on clinical-behaviour [6]).

Materials and methods

The study is based on samples of JNA obtained following surgical excision from 14 patients with age 14–23 years and the study was approved by Institution Ethics Committee. The number of samples for 7 molecular markers was different (14 for VEGF, FGF, c-Kit and c-myc while 13, 12 and 9 samples for Ras, PDGF and p53, respectively). The clinical parameters that were especially noted were age (pre-adolescent vs postadolescent), duration of symptoms, radiological staging (lateral extension vs skull base extension), intraoperative haemorrhage, tumor-volume/weight and recurrence. The imaging was staged as per Mishra staging criteria, as well as Radkowsky staging criteria while recurrence was defined as reappearance of the disease on follow-up but only after documented complete resection. The lab-methodology and molecular data analysis has been described in the earlier publication [4] but is briefed as follows.

The tumour samples obtained were snap frozen and stored at minus 80 degree centigrade in RNA litter. The analysis for molecular expression was undertaken at a Central Laboratory. The m-RNA expression of the marker as obtained through RT-PCR defined its molecular expression. Eight samples of histologically proven nasal polyposis were taken as control.

RNA extraction Approximately 100 mg of each tissue sample, as mentioned above, were homogenized by Kimble® Kontes Disposable Pellet Pestles. Total RNA was extracted by Trizol reagent (Invitrogen) as per standard protocol. The quality of the isolated RNA was determined by running an aliquot of the RNA sample on a denaturing agarose gel (1.5%) stained with ethidium bromide (EtBr) to see 28S and 18S rRNA bands. Five microgram of total RNA was subjected to reverse transcription using "Moloney murine leukemia virus (MMLV) reverse transcriptase" (Invitrogen) at 42 °C for 1 h. The reaction for synthesis of first strand cDNA was primed with random hexamer primer.

RT-PCR Real-time PCR was performed on the Roche® LightCycler480, using FastStart SYBR Green Master (Roche), according to the manufacturer's specification. The primer sequences for the study gene (marker) is mentioned in Table 1. In general, the conditions for the PCR reaction consisted of—(a) initial denaturation at 95 °C for 10 s; (b)

Table 1 Corresponding ranks of molecular expressions of 7 markers

Sample no.	Ras	VEGF	FGF	PDGF	c myc	c kit	p 53
1		1	3		1	11	8
2	4	4	6		4	7	9
3	5	7	9	7	6	3	2
4	6	5	7	11	14	14	5
5	11	10	5	2	10	8	3
6	3	8	1	1	3	12	
7	7	9	11	8	5	1	7
8	8	12	10	3	8	5	6
9	2	2	2	5	13	13	1
10	10	14	14	10	11	10	4
11	13	13	12	4	7	4	
12	9	6	8	6	2	2	
13	1	3	4	12	12	9	
14	12	11	13	9	9	6	

The respective ranks of different markers are shown across each sample. The blank spaces correspond with the absence of data. The ranks of the marker having a larger sample are adjusted when compared with the marker having a smaller sample size. This is done by omitting those values of larger sample which correspond to the blank spaces of the marker having lower sample and the former are then re-ranked for a one-to-one comparison with the latter. These adjusted ranks are not shown in the table

denaturation at 94 °C for 30 s; (c) annealing for 30 s at respective temperatures as mentioned in Table-1 followed by (d) extension at 72 °C for 1 min. The steps (b)–(d) were repeated for 40 cycles before the reaction was stopped with a final extension step at 72 °C for 10 min. The amplification was performed in duplicate for each reaction and the results were normalized to the actin gene expression level.

Analysis of RT-PCR data First, the reaction condition for the gene was standardized by running the reaction in Gradient PCR. Results were confirmed by selecting condition yielding single band after running the PCR product at 1.5% agarose gel. Subsequently, the marker studies through RT-PCR were considered. Critical point (CP) threshold value was estimated for each marker and beta-actin derived from respective samples and controls. A differential CT value was obtained for each marker against every sample by subtracting CP values of beta-actin from the CP values of the respective markers. Subsequently, double delta CT (ddCT) value was calculated for every observation. Subsequently, the power of analysis (2—ddCT) was calculated for each value of expression, followed by average of power to estimate the variation in expression. Finally, the fold action (or fold activation) was calculated for molecular expression and the average of which (i.e. fold average) was used for comparative analysis. The fold average was used for statistical analysis through Sigma Plot software. A comparison of mean expression of marker was undertaken with control using Mann–Whitney's *U* test ($p < 0.05$).

To compare the degrees of molecular expressions of different markers with one another, the rank-values for every sample corresponding to individual markers were defined. Accordingly the expression-levels were arranged in decreasing order and "rank-1" was allotted for the highest expression corresponding to that marker. Similarly, the subsequent ranks (2,3,4, and so on) were allotted for each and every marker in its decreasing order of expression (Table 2). Overall, the intention was to compare the rank-values rather than true values of different markers that otherwise was not possible with different normative values of controls. Spearman's rank correlation test statistic was applied to compare the ranks of all these 7 markers. The sum of square of the difference of the individual ranks of the 2 markers was estimated (d) and the spearman rank correlation coefficient was calculated as per: $\mathbf{r_s} = 1 - 6\ (d)/n^3 - n$ (where n = number of ranks for comparison) while $p < 0.05$ was considered significant. Since the sample sizes of individual markers was not similar, the extra samples of those markers (having larger sample sizes) not corresponding to the markers with lower sample sizes (p53, PDGF, Ras) were excluded and thus 'adjusted ranks' for samples of the markers with larger sample-sizes (FGF, VEGF, c-Myc, c-Kit) were defined. These were further correlated in the same way.

Furthermore, a descriptive comparison of clinical behaviour (especially age, duration of symptoms, radiological staging, intraoperative haemorrhage, tumor-volume/weight) corresponding to the samples showing enhanced molecular expression on the higher scale was attempted. In addition, an attempt was also made to identify the composite clinical characteristics corresponding to the molecular-interacting groups. The 'directly–inversely-significant' observations were further analysed in clinical perspective and accordingly the possibility of defining a composite phenotype was also tried. Finally, an attempt was made to introduce a preliminary molecular signature/classification of JNA. In addition, another loose criterion was undertaken for comparison in which the highest and lowest expressions of every marker was defined by first 4 ranks ('Highest') and last 4 rank ('Lowest') values, respectively. For example, in a sample of 14, the ranks 1–4 were defined as 'highest' while '11–14' ranks as 'lowest'. A probable relationship was arbitrarily defined if at least 3 of 4 'Highest' or 'Lowest' ranks of one marker matched with the 'Highest' or 'Lowest' categories

Table 2 Spearman's rank correlation coefficient $\mathbf{r_s}$ and p values for interaction of molecular expression of all markers estimated by Spearman's rank correlation test

	Ras	VEGF	FGF	PDGF	c myc	c kit	p 53
Ras		0.8132	0.7417	-0.1748	-0.0824	-0.4011	0.0952
VEGF	**p<0.001**		0.7622	-0.2307	0.0857	-0.3934	-0.1833
FGF	**p<0.01**	**p<0.01**		0.3006	0.1076	-0.5736	0.1333
PDGF	p>0.05	p>0.05	p>0.05		0.4685	0.1118	0.2857
c myc	p>0.05	p>0.05	p>0.05	p>0.05		0.4373	-0.6833
c kit	p>0.05	p>0.05	**P<0.05**	p>0.05	p>0.05		-0.2
p53	p>0.05	p>0.05	p>0.05	p>0.05	**P<0.05**	p>0.05	

Spearman's rank correlation coefficient $\mathbf{r_s}$ are shown on the right upper side above the diagonal arrow and p values are seen on the left lower side below the diagonal arrow

of the other. Such a probable association was labelled as 'direct' when highest and/or lowest of one marker matched with the highest and/or lowest of the other. Alternatively, association was labelled 'indirect' when 'Highest' of one matched with 'Lowest' of the other or visa versa. Furthermore, the 'Adjusted ranks' (in case of p53, PDGF, Ras) were also compared similarly anticipating a different outcome. No statistical test was undertaken for this 'observational' comparison.

Results

The individual ranks of the fold expression have been depicted in Table 1 against the respective JNA specimen. With dissimilar sample sizes, the ranks were adjusted while using rank correlation test statistic. For example, while comparing 9 samples of p53, only the corresponding 9 values of VEGF from the same patients were considered in analysis while the other 5 out of total 14 samples of VEGF were excluded.

Table 2 summarizes both the $\mathbf{r_s}$ values arising out of comparison of ranks, as well as their level of significance (p values). A one–one comparison of each and every marker with all permutation and combination was analysed after making the necessary adjustments (as indicated above) wherever necessary. The $\mathbf{r_s}$ values are shown above the diagonal-line while p values below it. It is important to note that of 21 possible interactions only 5 correlations were significant. These were appreciated between VEGF/FGF ($p < 0.01$), Ras/VEGF ($p < 0.001$), Ras/FGF ($p < 0.01$), FGF/c-Kit ($p < 0.05$) and C-Myc/p53 ($p < 0.05$).

Although comprehensive descriptions of clinical characteristics of the respective markers have been discussed in detail elsewhere [6], Table 3 presents a brief descriptive comparison of the basic clinical characteristics of individual markers showing enhanced expression on the upper scale. VEGF, FGF and Ras revealed a predilection for postadolescent presentation and enhanced intraoperative haemorrhage as compared to other markers. However, skull base involvement was seen more with higher expressions of Ras, VEGF and c-Kit. The sample size of recurrence is too small to make any meaningful comparison. A basic similarity can be appreciated in many of the clinical features amongst markers showing significant interaction and reader is encouraged to read a comprehensive analysis of clinical behaviour along the continuum of molecular expression [6].

Finally, the results of 'loose criteria' are worth mentioning even if not statistically relevant, only because such 'potential' interactions may be validated for significance in larger sample. Considering all 14 samples, 5-interaction patterns were appreciated (1) Inc Ras/Inc VEGF/Inc FGF; (2) Dec Ras/Dec VEGF/Dec FGF; (3) Inc FGF/Inc VEGF/Inc Ras/Dec c-Kit; (4) Dec PDGF/Dec c-myc; and (5) Dec c-Kit/Inc FGF. These were somewhat consistent with the significant-interaction-pattern but after adjusting sample size, 3 different-interactions were observed: (1) Dec p53/Inc c-myc/Inc c-Kit, (2) Inc p53/Inc PDGF/Dec c-myc, and (3) Inc Ras/Inc VEGF (No Inc FGF). Also c-Myc interaction was associated with PDGF; while an inverse relationship existed between FGF and c-Kit. Owing to their statistical irrelevance these are not discussed further.

Table 3 Clinical characteristics of molecular markers [6] showing enhanced expression on the higher side

Clinical characteristics	Ras	VEGF	FGF	PDGF	c myc	c-Kit	P53
Age (years)	20.25	19.75	20.75	17.75	17	16.8	18.25
Duration of symptoms (m)	15.75	16.25	7.75	3.25	13.5	5.3	11.5
Intraoperative bleeding (ml)	1137.25	1362	1003.5	372.25	956.25	823	904.75
Tumour volume (ml)	25	23.5	23.25	20.75	30.25	32.8	33.25
Skull base extension	++	++	+	−	+	++	−*
Lateral extension	−	−	−	+	−	−	+
Recurrence	1/3	1/3	1/3	0/3	0/3	2/3	1/2

This table does not compare the features for cases showing molecular expression on the lower scale. The average of age, duration of symptoms, bleeding-amount and tumour size can be compared. With only 4 cases to compare the statistical significance cannot be established meaningfully. Second, the tendency for preferential extension is based on valid staging systems (see text) while recurrences are far too few to make a meaningful comparison. Overall the table provides a descriptive comparison only

*Associated with lower levels; '+' as per our observation indicates a positive preferential extension in 3 of 4 cases while '++' indicates a more strong positivity (presence of positive preferential extension in all cases). On the contrary "−" indicates no positive preferential extension and '+' indicates that positive preferential extension in 1–2 cases

Discussion

Five different subsets of statistically significant molecular interactions were identified in our population viz. (1) FGF/VEGF ($\mathbf{r_s}$ 0.7622, $p < 0.01$); (2) Ras/FGF ($\mathbf{r_s}$ 0.7417, $p < 0.01$); (3) Ras/VEGF ($\mathbf{r_s}$ 0.8132, $p < 0.001$), (4) FGF/c-Kit ($\mathbf{r_s}$ −0.5736, $p < 0.05$); and (5) c-Myc/p53 ($\mathbf{r_s}$ −0.6833, $p < 0.05$). These may reflect the first 'molecular signatures' in JNA and accordingly may suggest a preliminary 'molecular classification'. No study till date has described such interactions but these need to be further validated. However, it could not be established whether the relationship was direct or inverse. In this regard, the loose criterion for comparison reflected this nature but these at present are only speculative. Overall, the most outstanding observation in our study was the implication of FGF/VEGF/Ras interaction in JNA and that too having a direct correlation. Since the expression of FGF, VEGF & Ras was up-regulated in all JNA samples, this marker-complex with enhanced significance (see *p* values), can be considered as a very consistent molecular target in JNA. However, these pathways are very complicated and basic mRNA analysis, even with controls, may not provide sufficient data to support our claim, but this in fact is the best such evidence today.

The enhanced expressions of VEGF, FGF and Ras revealed a similar trend in clinical behaviour regarding age, intraoperative-haemorrhage, tumour-volume, skull-base extension, tendency for lateral-extension and recurrence-potential criteria (Table 3). Hence a clinical and molecular correlation of VEGF/FGF/Ras complex can be speculated whereby the enhanced intraoperative haemorrhage and skull-base extension may be regarded as surrogate markers of VEGF/FGF/Ras complex dominance. Many other interesting observations were noted. The c-Kit/FGF interaction is also reflected by enhanced tendency for skull-base extension and a more recurrent potential. Furthermore, c-Myc/p53 interaction reveals inverse relationship in terms of extension. Accordingly, upper expressions of c-Myc and lower expressions of p53 were associated with skull-base involvement while the opposite expressions were appreciated with lateral-extension (Table 3).

The main strength of this study involves estimation of the real (m-RNA) expression of 7 markers in a single cohort. However, inclusion of proteomics would have further elaborated the cascade. There are other limitations of this study as well. First, this involves a small sample size that, however, can be justified for this rare disease specially after considering the sample sizes cited in other past studies. Second, further limitation in samples of Ras, PDGF and p53 is likely to further dilute the comparative significance. Third, the observations based on the loose-criteria seem to have a very little relevance unless validated statistically. Finally, a clinical comparison of lower expressions has not been considered since the idea was to compare higher expression with clinical behaviour. In absence of the literature on molecular signature, these interactions may provide a lead for further investigations in JNA.

A few speculations for molecular targeted therapy can be made based on our supporting data. With all the three markers showing significant interaction with each other (*p* values of < 0.001, < 0.01, < 0.01, Table 3), the authors feel that targeting any 2 markers of this VEGF/FGF/Ras complex is likely to down-regulate the third and thus may have an overall regressive effect. The relevance of FGF/c-Kit ($p < 0.05$, Table 3); and c-Myc/p53 ($p < 0.05$, Table 3) interactions may be important when targeting VEGF/FGF/Ras complex per se achieves unexpected response. The following section presents the relevant evidence supporting our significant molecular interactions.

Interaction between VEGF and FGF

The VEGF and FGF are the most potent angiogenic growth factors associated with high vessel densities [7] in JNA. Both activate tyrosine kinase (TRK) and may modulate through common ERK pathway. FGF signalling modulates tissue fibrosis [8], promotes endothelial proliferation [9] and as known for angiogenesis has been implicated in JNA [10]. VEGF stimulates apoptosis under ischemic conditions [11] and has also been shown to modulate transcription of members of forkhead family (FKHR, FKHRL1, AFX) expressed by endothelial cells via PI3K/AKT signalling [12]. PI3K/AKT signalling is implicated in the development of vasculature/structurally abnormal blood vessels [13].

A significant cross-talk seems likely between VEGF and FGF in context of angiogenesis, with VEGF appearing earlier than FGF during angiogenesis [14]. The angiogenesis was seen to occur rapidly on addition of both VEGF and FGF-2 together to endothelial cells than when added separately, suggesting a synergistic interaction [15]. The induction of angiogenesis induced by FGF-2–has been shown to be partly dependent on VEGF-activation and presence of endogenous VEGF-C [16]. Evidence for synergistic action between VEGF and FGF-2 has also been demonstrated in xenograft models with tumor cell transfectants. Simultaneous expression of both FGF-2 and VEGF caused faster growing lesions with enhanced blood vessel density and permeability [17]. The inhibition of FGF-2 production was associated with a significant decrease in blood vessel density and size, while VEGF inhibition accompanied a decrease in pericyte organization, vessel patency, and permeability. Hence FGF-2 and VEGF do stimulate angiogenesis

synergistically but have different effects on vasculature. FGF-2 is likely to modulate VEGF pathways indirectly as well. For example, in breast cancer cell line (T47D), FGF-2 activated hypoxia-induced VEGF release through PI3K pathway, and HIF-1a expression [18]. Such an extensive cross-talk and synergism can be expected in JNA as well but only a single study [7] till date has reported their enhanced expression in JNA simultaneously. Neutralizing monoclonal antibody to VEGF inhibits FGF-2-induced endothelial cell proliferation and angiogenesis [19] while FGF may play a crucial role in the resistance to anti-VEGF therapy as well. Targeting PI3K/AKT/PTEN as a common pathway for both VEGF and FGF may be important in inhibiting angiogenesis in JNA as has been proposed in leukemia [20].

Interaction between Ras and VEGF

Ras regulates normal cellular proliferation by at least 10 downstream-signalling pathways [21]. It is required for the induction of the angiogenic phenotype in response to VEGF that involves PI3K/AKT, and Erk, signalling. A possibility exists that chronic Ras activation in endothelial cells may be sufficient to promote angiogenesis and development of vascular anomalies [22]. However, no evidence suggests the role of Ras in JNA and only a single study has refuted any such association [23]. On the contrary our JNA population has shown a very significant enhancement of Ras in all the cases [4]. A role of Ras in angiogenesis was suggested [24] in a mouse mammary carcinoma model. The migrating fibroblastoid cells were under the effect of Ras induced VEGF expression and angiogenesis but interestingly overexpression of VEGF in non-Ras-transformed epithelial cells was not sufficient to promote vascularization *in vivo*. Moreover, mevastatin, an inhibitor of Ras activation, completely blocked VEGF expression in cultured primary endothelial cells [25]. Meadows [26] also showed that inhibition of Ras signalling limited the activation of ERK, further suggesting that inhibition of Ras may be anti-angiogenic. Our JNA population is molecularly 'favourable' in terms of showing a remarkable increase in expression of Ras, and thereby revealing a potential 'soft target'.

Interaction between Ras and FGF

Yamada & Yoshimura [27] described a Computer Modelling of Ras-MAPK Signal Transduction Pathway. EGF is known to induce cell division, while FGF differentiation and more Ras activation is likely through FGF pathway since EGF receptors are less abundant. Klint et al [28] too have agreed that in FGF stimulated endothelial cells, Ras pathway is initiated via Shc or FRS2 and the MAPK Erk2 is thus activated in a sustained manner. Furthermore, it has been mechanistically demonstrated that FGFR1 activation by FGF-2 elicits distinctive Ras (and PKCδ) pathways, which concertedly trigger MAPK signalling to mediate biological effects of FGF-2 [29].

Interaction between c-myc and p53

The up-regulation of c-myc is nonlinear [30] although the gene acts as the "principal regulator" of cellular metabolism/proliferation and apoptosis in selected conditions. Only 3 studies have reported the status of c-Myc in JNA with inconsistent results. Our population does not reveal c-myc as a surrogate marker for any specific clinical phenotype per se, indicating a gross redundancy in the interactive pathways. Deregulation of c-myc is common in malignancies [31] and a close crosstalk between beta-catenin, androgen receptor (AR), and c-myc is suggested but such crosstalk does not appear to exist in our JNA population. On the other hand, of the 2 studies on p53 in JNA one reported increased mRNA expression in 32% [32], while the other revealed losses of p53 gene in 5 of 7 cases without mutations [33]. Although p53 is usually down-regulated in cancer, its up-regulation in our population [4] may reflect some oncogenic stress to enhance expression of its target genes. The crosstalk between p53 and c-myc is well known, and therefore, may have an implication in JNA as well. The downregulation of c-myc by p53 may also contribute to its tumor suppressor function and controversy still exists whether it is under a direct transcriptional control or indirectly mediated by DNA damage or as suggested [34] by transcription through histone deacetylation. A state of confusion exists regarding the effect of c-myc downregulation by p53 on apoptosis and proliferation. While one study suggests tumour formation to correlate with the loss of apoptosis, rather than an increase in proliferation [35]; the other study reveals the reverse findings [36]. It is yet unknown as to what mechanism operates in JNA but in the context of apoptosis in cancer, p53-Bax regulatory pathway is related to c-Myc, and proapoptotic signals produced by c-Myc and p53 may act at several levels. The most cited model linking c-Myc over-expression to the p53 pathway is via transcription induction of ARF by c-Myc, which in turn inhibits Mdm2 (a key negative regulator of p53) [37]. Phesse et al [38] have demonstrated in vivo a crucial role of endogenous c-Myc in signalling apoptosis (induced by DNA damage) through modulation of p53 suppressor protein. Furthermore, the miRNAs may also play a role in the p53-mediated posttranscriptional regulation of c-Myc [39].

Interaction between FGF and c-Kit

Activation of c-Kit pathways leads to the release of VEGF & FGF, and it is likely that both FGF and c-Kit being TRK agonists may share a part of same downstream pathway

responsible for angiogenesis. Implication of c-kit in JNA on the other hand is suggested by only 2 of 3 studies. In one study, a strong immunohistochemical expression of c-kit in the stromal cells of JNA samples has been demonstrated [40], while the other study failed to find any expression [41]. However, our JNA population has shown an overall significant enhancement of c-kit expression [4]. Much of the interaction between FGF and c-Kit has been studied in erythroid cells and such interactions related to cell differentiation/proliferation may be applicable in JNA as well. The c-kit expression has been shown to decrease with cell differentiation [42]. Hence the expression of c-kit may reflect the maturation/differentiation index in JNA accordingly. Low levels of bFGF inhibits erythroid differentiation while bFGF alone increases expression of c-kit and promotes a primitive phenotype in K562 cells [43]. Furthermore, FGF-9 in a mice model is shown to have a potential of activating c-Kit progenitor cells, and thereby enhance angiogenesis/neovascularization [44]. Such interactions can be speculated in JNA and still need to be proven.

Many already known interactions in other tumors were not appreciated in our study such as Ras with p53 [45], Ras with c-Myc, c-Kit with VEGF, etc. Implication of interaction of PDGF with c-Myc (loose criterion) still needs statistical validation. Moreover, our institutional observations suggest an evolving role of oncogene Ras [4] and human papilloma virus (HPV) [5] in etiopathogenesis of modern JNA. This may be a possible reason to explain the rapidly rising incidence of JNA in our population [1] as well as suggesting the evolution/dominance of neoplastic etiology. A particular importance of molecular targeting lies in managing those high-risk cases (orbital apex/skull base/intracranial extensions and recurrence prone) that accompany high surgical morbidity or a poor quality of life thereafter. Although this is the dawn of infancy, a large multicentric trial is further likely to elaborate the molecular classification of JNA.

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Compliance with ethical standards

Conflict of interest All authors declared that they have no conflict of interest.

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