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Review Article

Molecular Biology of Intracranial Aneurysms and their Clinical Implication

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Abstract

Background: Intracranial aneurysms are a typical lesion ranging from 1% to 5% and causing frequently subarachnoid hemorrhage with high rates of morbidity and mortality. With this devastating scenario, efforts must be directed to improve diagnosis and treatment. Molecular biology of intracranial aneurysms advanced a lot in the last years, improving the understand of their etiology, natural history, and especially, potential targets to be explored in prevention and treatment. In this study, we briefly review the molecular biology of intracranial aneurysms and their potential clinical implications.

Review: In the field of genetics, studies have identified some specific loci in susceptible genes correlate with cell cycle and endothelial function leading to formation of IA. Among them, the gene that appears to be more correlated with formation of IAs, is the elastin gene, located on chromosome 7q11. Others include chromosomes 8q and 9p, associated with a higher risk of IA in cigarette smoking patients. Familial genetic studies reveal that multiple genes must be considered to the etiology of IA. Inflammation seems to be related to the pathogenesis of IA. Several mediators of inflammation (leukocytes, complement, immunoglobulins, cytokines, and other humoral mediators) have been analyzed as contributors to the genesis of IA, and new data suggests that therapies targeting to inhibiting inflammatory cascades could have efficacy in the IA treatment. Metalloproteinases, beyond being candidates as biomarkers for IAs, are also promising therapeutic targets. Imidapril, an ACE (angiotensin-converting enzyme) that acts as a potent inhibitor of matrix metalloproteinase-9 (MMP-9) significantly decreases the size and medial thinning of induced IA. Finally, circulating neutrophils have RNA expression that carries an IA signature promoting potential use to identify patients with IAs, highlighting the possibility to use peripheral blood samples as predictive biomarkers to diagnose IAs before its rupture.

Conclusion: Ruptured IAs remains a catastrophic disease. Efforts to prevention, diagnosis, and treatment must orient our clinical practice. With the recent advances in the field of molecular biology, there is an expectation on the development of target-directed therapy for intracranial aneurysms. Besides, diagnostic and preventive measures using potential biomarkers and genetic screenings may change the natural history of IAs.

Keywords: Intracranial Aneurysm; Subarachnoid Hemorrhage; Gene; Molecular Biology

Abbreviations

SAH: Subarachnoid Hemorrhage; IA: Intracranial Aneurysms; UA: Unruptured Aneurysms

Introduction and Background

Intracranial aneurysms (IA) are common lesions ranging from 1% to 5% and constitute a significant etiology of subarachnoid hemorrhage (SAH) resulting in high rates of morbidity and death. In this devastating scenario, efforts must be directed to improve diagnosis and treatment. Molecular biology of IAs advanced a lot in the last years, improving the understanding of their etiology, natural history, and mainly, potential therapeutic targets. In this study, we shortly review the molecular biology of IAs and its potential clinical implications.

Aneurysmal SAH involves catastrophic hemorrhage with dismal outcomes. The mortality and morbidity rates are exceedingly high [1]. Prevention and early treatment are the best strategies to improve clinical results. Biomolecular studies may help to delineate policies that allow early diagnosis in population in risk [1]. The most important epidemiological risk factors correlated with the development of IAs are older age, cigarette smoking, alcohol consumption, sex hormones and the presence of systolic hypertension. Heavy smokers, light and former ones are 11, 4 and 8 times more prone to the development of IA than nonsmokers. [4,5] There is not known predictive tools with epidemiological power to identify individuals with a substantial higher risk to make pre-symptomatic screening by current imaging techniques cost-effective. Possibly, epidemiological factors are contributing with the development of intracranial aneurysms, specially impacting on the preprogrammed molecular environment of vascular tissue. [1,6]

Review

Genetics factors

Actually, concerning about IA genesis or bleeding we cannot identify one single gene as causative. If the mechanism was clarified, we would develop therapy targeted, preventing its rupture. [6,7] The identification of a genetic marker associated with an increased risk of formation and rupture of an IA, undoubtedly will facilitate the screening and decision treatment of IA. Although a familial predisposition is the most substantial risk factor for the development of IA, the correct mechanism of inheritance is uncertain. Twin and familial genetic studies reveal that relatives in affected IA families show earlier onset of disease and are up to seven times more likely for IA rupture and SAH. [4] Multiple genetic susceptibilities are, hopefully, considered to cooperate in the etiology of IA. [6] Some epidemiological studies revelas a strong genetic influence: siblings have a six-fold increased risk of develop being the same disease. To notice, the risk is three to seven times higher in first-degree relatives of patients with SAH when compared with the general population. [6] Some strategies are emerging to identify genetic risk factors and clarify the molecular pathobiology of IAs, like genome-wide approaches such as DNA linkage and genetic association studies, as well as microarray-based mRNA expression studies. [8,9].

Both family-based genetic linkage studies are required for the full understanding of the genetics of IA and the two significant approaches are the linkage approach locating the locus of the disease using families and the association approach (direct or indirect) identifying a potential disease allele in a case-control design.[6]

In patients that smoke was located spots on chromosomes 8q and 9p that can be related with the risk of development of IA significatively high. [8]

Although the molecular basis of the disorder is not known, family studies strongly support genetic factors in the formation of IAs. According to many studies there are identified some chromosomal loci showing suggestive evidence of linkage specially in familiar cases. The mode of transmission for harboring an IA was not discovered, and the genetics of the disorder appear to be complicated, involving multiple loci and the interaction of numerous genes. Two regions were localized on chromosome 7q and 19q in both samples of Japanese and white patients. Other studies interpretation might lead to the identification of genes or pathway that are important for IAs pathogenesis. Nahid et al. detected a single locus in chromosome 1p34.3–36.13 in a study of a large family with IA (six living patients and four deceased). [6] Up to 25% of IAs can harbor genetic factors explaining it, as showed in some genetic epidemiological and statistical analysis and what is most interesting is that, of these, only 5% are associated with heritable connective tissue disorders, and the remaining 20% are not. [4] Diseases of the extracellular matrix (ECM) of the vessels

probably are risk factors in the genesis of intracranial aneurysms. In patients with ruptured IAs was observed some reduction in structural proteins of the ECM such as arterial defects detected in the skin or even in intra- and extracranial arteries samples of patients with IA. The best candidate genetic loci for IAs include genes coding for structural proteins of the ECM of the arterial wall. [10,4] In a Dutch study, it was found six single nucleotide polymorphisms (SNPs) that were associated with IA: serpine1, transforming growth factor beta induced (TGFBI), perlecan (HSPG2), fibronectin (FN1), fibrillin 2(FBN2) and alpha 1 type IV collagen (COL4A1), signaling that the failure of maintenance of the integrity of the ECM of the arterial can confer susceptibility to intracranial aneurysms. [10,4]

Unfortunately, no single gene has been recognized as a candidate gene to explain IAs. Probably, the elastin gene, which is located on chromosome 7q11, appears to be a highly likely candidate involved in the formation of IAs, because it was found in both Japanese and white patients from Utah (7q11).[4] The region most susceptible to explain IAs formation is in chromosome 11q and as previously suggested in different previous studies. Probably the most important step toward creation of new therapeutic and diagnostic measures to IAs will be when proteins involved in it is formation could be identified [6,7].

Studies showing addressing genes (DEGs) of unruptured IA differentially expressed provide additional data for early diagnosis and treatment. Microarray analysis identified the expression of the hub protein MYH11 and its co-expression genes ACTA2, MYLK, and MYL9, along with their transcripts, and probably can create tools for early diagnosis and treatment of IAs. [11,12]

The genetic risk prediction tests are currently unfeasible, even larger studies as the recent genome-wide association study of IAs in Finnish, Dutch and Japanese cohort including 5,891 cases and 14,181 controls could explain only up to 5% of the familial risk of IAs. [8]

Another study using mRNA expression profiles of human IAs were obtained from the GEO database for integrated analysis. DEGs were screened, and functional annotation of DEGs (differentially expressed genes) was conducted. The IAs-specific transcriptional regulatory network was constructed. In the IAs-specific regulatory system, top ten TFs (transcriptional factor) covering the most downstream DEGs were NFIC, NFATC2, FOXO3, ZNF354C, ZNF263, BRCA1, FOXL1, NR4A2, SP2 and EGR1, which may cooperate and play roles in the pathogenesis of IAs. Moreover, the target genes (OIT3, CNTN5, PLA2G12B, and TMPRSS4) could be genes of interest in IAs, and dysregulation of them may be closely associated with IAs pathology. [12]

Toward understanding molecular pathogenesis of diseases, we could develop new treatment strategies. Identifying susceptible genetic alterations may lead not only to understand the mechanism of formation and rupture of IA but also to help discover and develop the potential effective pharmacological therapy. These clearly demonstrate how far from understanding the pathogenesis of IAs an how we need efforts and investments for extensive genetic research and its potential for future prevention. [4] We hope, some of these identified genes may be good candidates for molecular markers of rupture-prone IA and for therapeutic targets to prevent its rupture. [13]

Role inflammation

The nervous system is immunologically active where various constituents of the immune and inflammatory response, like leukocytes, cytokines, adhesion molecules, immunoglobulins (Igs), and complement (C) are found working harmonically. Inflammation seems to be related to the pathogenesis of IA [2,14] because it's fundamental to the healing, however excessive Inflammation disbalance repair and degradation of the wall and can cause IA rupture. [13,3,5]

Endothelial dysfunction results in pro-inflammatory phenotype leading to IA formation and development. When the cellular constituents of the vessel wall are injured the result is apoptosis demonstrated by the finding of degradation of endothelium and smooth muscle cells what can explain aneurysmal formation, progression, and eventual bleeding. Several mediators of Inflammation (leukocytes, complement, immunoglobulins, cytokines, and other humoral mediators) have been analyzed as contributors to the genesis of IA demonstrated by different profiles of gene expression compared with control arteries related to immune inflammation/response. Vessel infiltration wall by macrophages and T cells associated with aneurysm rupture was reported in a study that histologically compared 42 ruptured with

24 unruptured IAs. Furthermore, preliminary data suggest that therapies targeting the inflammatory response may have efficacy in the IA treatment. [2,3,16]

Multiple cytokines are involved in the pathogenesis of IA and Inflammation especially IL1b, IL6, tumor necrosis factor-a (TNFa) and nuclear factor-kappa B (NF-κB) and tumor necrosis factor-alpha (TNF-α) acclaimed as potentially crucial molecules in the inflammatory process. NF-κB is a transcription factor that is known to be closely related to Inflammation and is activated in endothelial cells at the site of arterial bifurcation in the early stages of aneurysm formation because of the induced hemodynamic stress. Particularly, expression in aneurysm walls of mRNA for TNF-α has been observed in humans. Regarding cerebral vasospasm some inflammatory metabolites can be found in the first days after SAH before its appearance such as arachidonic acid and its metabolites, prostaglandins, prostacyclins, thromboxanes, and leukotrienes, closely related to complications of IA bleeding. [17,3,18]

Another interesting finding was the role of Angiotensin II (Ang II). It was founded in mice studies that Ang II stimulates vascular inflammation, oxidative stress, formation, and rupture of intracranial aneurysms. More interesting is the fact that Ang 1–7 acts on Mas receptors and generally counteracts harmful effects of Ang II decreasing formation and rupture of intracranial aneurysms. [19,20]

Potential biomarkers

With better understanding of the pathophysiology of IA we can discuss potential biomarkers that could help or clinical practice with better understanding of why IA occur and promoting early diagnosis and treatment avoiding its rupture and minimizing its complications for benefit of our patients.

IA diagnosis, rupture and complications

The finding of elevated serum levels of Metalloproteinases (MMP) is a potential biomarker for IAs because MMPs promotes the breakdown of extracellular membrane (ECM), an important step in the pathogenesis of IAs. [4] Moreover, the levels of MMP 2 and MMP 9 were found to be higher in ruptured compared with UA in a series of 30 patients, suggesting that excessive breakdown of vessel extracellular matrix eventually leads to rupture. [2]

Curiously IAs are stabilized when the expression of TNF-α is reduced, or expression of anti-inflammatory cytokines increases, however continuous expression of TNF-α induces aneurysmal rupture, being these cytokines potential targets to reduce IAs rupture.[3]

Examination of patients with connective tissue disorders give us clues to understand IAs formation and behavior. Of them the most studied was Ehlers Danlos Syndrome (EDS), for instance skin biopsies taken from IA patients without known connective disease encountered repetitive aberrations of collagen fibrils and elastic fibers that under electron microscopy were very similar to those typically observed in EDS patients. EDS abnormalities can be classified into two groups: type III EDS-like (hypermobility), and type IV EDS-like (vascular type). The classical finding of EDS IV is a rupture of large vessels due to mutations in type III collagen disrupting the structure of the collagenous network and the properties of the adventitial layer.[4] This reduction on collagen type III eficiency was found to be associated with formation of IAs. Another lesions of IA patients promotes a reduced ratio of type III/type I collagen demonstrating its role in IAs formation and progression. [4]

An interesting finding is the higher plasma elastase (PE) seen in IA patients. Serum levels of a-1 anti-trypsin (AAT), an elastase-specific inhibitor, are significantly lower in IA patients with SAH than in controls and more promising, plasma elastase/AAT ratios demonstrate the activity of elastase and are two folds higher in the serum of IA patients than in the serum of controls, allowing that following the curve of this metabolites we can predict IA rupture. [4]

Analysis the pro-inflammatory and vasoconstrictive properties of the cerebrospinal fluid (CSF) after aneurysmal subarachnoid hemorrhage (SAH) in vivo and in vitro found a linear relationship with the number of rolling and stinking leucocytes with decrease arterial diameter and vasospasm compared with controls. [17] As a fibrin degradation product, d-dimer values were found to be elevated in both serum and cerebrospinal fluid (CSF). It has been suggested as a biomarker for the increased risk for poor outcome after SAH. [4]

After IAs rupture some interleukins can be found, especially Interleukin-6 (IL-6). And interesting finding is that IL-6 relation

in CSF and serum is a stronger predictor of outcome (ρ = 0.721; P<0.001). High levels of IL-6 in the CSF or in the plasma were directly correlated with poor outcome at 30-day mRS (modified Rankin scale) (OR, 17.97; 95% CI, 1.51–214.33; P = 0.022), (OR, 12.71; 95% CI, 0.90–180.35; P = 0.022), respectively. The level of IL-6 in both plasma and CSF were an independent prognostic biomarker that may support to identify patients with a risk of neurological poor outcome after SAH for IAs. [18]

Another result concerning IAs progression and/or rupture is of macrophage infiltration in aneurysm wall. The turbulent flow induces expressions of chemoattractant and adhesion molecules for macrophages such as MCP-1 (monocyte chemoattractant protein-1) in endothelial cells, during IA formation/progression. [15,20] An initial study using vessel wall MRI (with standard gadolinium-based intravascular contrast agents) has concluded that aneurysm wall contrast enhancement is more common in ruptured aneurysms than in unruptured aneurysms and more interesting is when, ferumoxytol, a drug containing iron oxide nanoparticles, which labels the phagocytic activity of macrophages, were used to detect these macrophages in the aneurysm wall, in other words inflammation, on iron-sensitive MRI pulse sequences. [20].

Another potential biomarker for IAs diagnosis is RNA expression from circulating neutrophils that carries an IA- associated signature.[15]

These techniques are still very expensive and the search for low-cost strategies such as blood testing may offer a reliable and accessible diagnostic alternative. [15]

Prevention and treatment

In an experimental study in rats, imidapril, an ACE (angiotensin-converting enzyme) inhibitor and a potent inhibitor of matrix metalloproteinase-9 (MMP-9) significantly suppressed the size and medial thinning of induced IAs. The expression and activity of ACE were not induced in IA walls. Furthermore, imidapril did not affect the ACE activity and expression, suggesting that the inhibitory action of imidapril was independent of inhibition of the RAS. Imidapril inhibited MMP-9 activity upregulated in IA walls. Besides, imidapril suppressed MMP-9 activity in a dose-dependent manner.

An experimental study demonstrated that the infusion of Ang 1–7 attenuated ruptured IA and mortality in a mouse model of IA. Ang 1–7 did not decrease the expression of inflammation markers but regulated the expression of MMP-9 and cyclooxygenase-2. The study shows a potential novel therapeutic strategy for medical IA management. (19,20)

Therapeutic administration of a TNF-α inhibitor significantly reduced aneurysm formation in rats. [3] Medical treatments inhibiting inflammatory cascades in IA development are likely to prevent IA progression and rupture. [16]

Statins and aspirin are expected to suppress IA progression by their anti-inflammatory effects and analyzing critically the International Study of Unruptured Intracranial Aneurysms (ISUIA) this potential becomes clear. Patients with unruptured IAs who used aspirin (acetylic acid) since before the study had a lower risk of bleeding compared to non-users. Aspirin exerts its antiplatelet and anti-inflammatory actions by inhibition of COX-1 and -2. More interesting, other studies demonstrated that after three months of treatment with aspirin, the signal intensity corresponding to the uptake of ferumoxytol by macrophages in the IA wall was attenuated, confirming the anti-inflammatory effect of aspirin on the IA wall. (16)

Decoy oligodeoxynucleotides (ODN) inhibiting inflammatory transcription factors such as nuclear factor kappa-B (NF-κB) and Its-1 are the likely choice of the prevention of IA development. The treatment with NF-κB decoy ODNs caused a dramatic decrease in the inflammatory response and IA incidence in rats but only when started early. [16]

Another interesting finding in the pathophysiology of IAS is the role of estrogen. Estrogen decreases some of the inflammatory molecules that could cause IAs explaining why in menopause the incidence of aneurysm increases. [8,20] More interesting is that the risks of SAH are decreased in women whose first pregnancy is at an older age and women who have ever used HRT (hormone repository therapy) but not OCPs (oral contraceptives). These findings indicate an independent role for hormonal factors in the pathogenesis of aneurysmal bleeding and may support a protective role for HRT on the risk of SAH in postmenopausal women. [5]

Conclusion

Ruptured IAs remain a catastrophic disease. Efforts to prevention, early diagnosis, and treatment must orient our clinical practice. Molecular biology of intracranial aneurysms advanced a lot in the last years, improving the understand of their etiology, natural history, and especially, allowing potential targets to be explored in prevention and treatment. Potential biomarkers are necessary for early diagnosis and treatment, which may change the natural history of IAs.

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