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Single Nucleotide Variants and Somatic Aberrations of A20 in Immune-Related Diseases and Lymphoid Neoplasms

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Abstract

A20- also called tumor necrosis factor (TNF) α induced protein 3 (TNFAIP3) - is an ubiquitin-modifying enzyme, which acts as negative regulator of the NF-κB pathway. Aberrant activation of NF-κB has been shown to contribute to chronic inflammation and development of cancer. Somatic mutations and epigenetic silencing at *A20* have been recently identified to be involved in the development of classical Hodgkin and many other types of non- Hodgkin lymphomas, thereby rendering A20 a classical tumor suppressor. Moreover, single nucleotide polymorphisms of *A20* have been described to participate in the development of autoimmune diseases of the intestinal tract, systemic lupus erythematosus, rheumatoid arthritis and diabetes mellitus Type I. Here we want to summarize different polymorphisms related to autoimmune disorders and mention other SNPs, mutations and epigenetic mechanisms affecting *A20* in lymphomas and multiple myeloma.

Keywords: A20; NF-kB; Single nucleotide polymorphisms; Somatic aberrations; Epigenetic silencing; Autoimmune disease; Lymphoid neoplasms

A20 Structure

A20, also known as tumor necrosis factor (TNF) α induced protein 3 (TNFAIP3), is an ubiquitin-modifying enzyme, which acts as negative regulator of NF-κB. The protein was first identified in 1990 as a cytokine response gene in human umbilical vein endothelial cells [1]. *A20* is located on chromosome 6 and has eight coding exons [2]. As shown in Figure 1 the C-terminal region contains seven zinc finger (ZnF) domains, whereby domain 4 harbors an ubiquitin-binding and E3 ligase activity and ZnF 7 has an additional ubiquitin-binding activity. The N-terminal region is characterized by an ovarian tumor domain (OTU) responsible for deubiquitinylation. The deubiquitinating (DUB) activity is thereby mediated by a catalytic cysteine residue at position C103 of A20 [3,4]. Importantly, *A20* induction can be mediated by various nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-mediated stimuli through NF-κB binding sites in the *A20* promotor region and functions itself as a negative regulator of the same pathway thereby mediating a regulatory crosstalk [5].

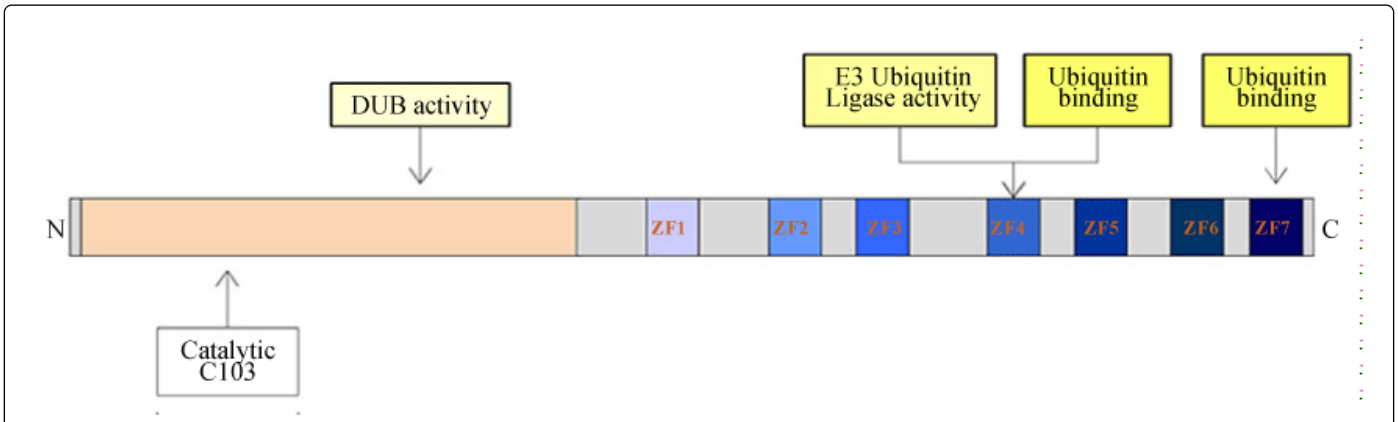

Figure 1: Schematic representation of the A20 protein structure - adapted from Ma et al. [4] At the N-terminal site the A20 protein has an ovarian tumor domain (OTU) harbouring a deubiquitinating (DUB) activity, which is mediated by a cysteine residue at position C103. At the C-terminal domain the protein contains seven zinc finger (ZnF) regions with an E3 ubiquitin ligase activity on zinc finger 4 and ubiquitin-binding regions lying on ZnF 4 and ZnF 7, respectively.

A20 as a negative regulator of the NF-κB pathway

A20 is mainly regulated by the CARMA1-BCL10-MALT1 complex, which connects T-cell receptor signalling with the canonical NF-κB pathway [3]. The way by which A20 inhibits NF-κB signaling is very complex and unique. Like other E3 ligases and deubiquitinating enzymes, A20 is involved in ubiquitinylation of several proteins [6]. The polyubiquitinylation process represents a posttranscriptional modification, which causes proteasomal degradation. The OTU domain of A20 cleaves lysine-63 linked polyubiquitin chains from the receptor-interaction protein 1 (RIP1), which is an essential factor for the tumor necrosis factor (TNF) receptor 1 (TNFR1) signaling pathway. This reaction is followed by adding lysin-43 linked polyubiquitin chains and subsequent degradation of RIP1 through the E3 ligase activity of A20 [5]. This suggests that A20 has a double function in the TNFR1 pathway [7]. Furthermore, A20 does not only regulate the ubiquitinylation of RIP1 in the TNFR1 pathway but it also suppresses the activation of NF-κB through TNF by deubiquitinating TRAF6 [8,9].

A20 and autoimmune diseases

One of the most important and best-characterized pathways involved in innate and adaptive immunity and tumorigenesis is the canonical NF-κB pathway, whose constitutive activation leads to pro-inflammatory processes. A20 is induced by NF-κB itself and functions upon induction as an auto-negative feedback regulator of the pathway. With respect to its important regulatory role constricting overwhelming immune responses, it has been identified as a susceptibility gene for multiple inflammatory processes as well as autoimmune diseases. It is assumed that single-nucleotide polymorphisms (SNPs) of *A20* favor pro-inflammatory processes through its reduced general anti-inflammatory potential. The SNPs affecting *A20* and being involved in autoimmune diseases are distributed throughout the whole protein sequence, thereby affecting the N- and C-terminal domains as well as coding and non-coding regions of the latter, leading to reduced function and/or expression of A20. Several genome-wide association studies (GWAS) and other smaller genomic studies identified several distinct polymorphisms of *A20* located on the human chromosomal region 6q.23. Generally, polymorphisms of *A20* seem to be important in intestinal homeostasis and mucosal biology of the gut. NF-κB and thus A20 are key players in mucosal integrity participating in control of inflammatory processes and inflammatory cytokines secreted by resident immune cells. Evidence for a role of A20 in intestinal biology comes from genomic studies of different populations, where polymorphisms of *A20* have been shown to be implicated in inflammatory bowel disease (IBD), Crohn's disease and coeliac disease. Genomic analysis of Caucasian and British population showed a reduced or defective function of A20 in IBD and Crohn's disease [4,10-16]. Furthermore, SNPs in the *A20* locus have been identified to be predisposing for the development of other autoimmune disorders like rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). In RA excessive secretion of proinflammatory TNFα plays a key role in disease progression and TNFα antagonists serve as therapeutic targets for the treatment of this autoimmune disorder. SLE patients represent reduced self-tolerance and increased interferon levels. Both diseases share -at least partially- polymorphisms affecting the same nucleotides. Two SNPs, rs2230926/ F127C and rs5029941/ A125V, both lying in exon 3 in the OTU domain, have been shown to be important in SLE. A125V has been associated with increased risk of IBD in African-Americans, whereas it seems to have a protective function in SLE [17], while F127C has been identified to negatively affect the inhibitory capacity of A20 in SLE. Moreover, the SNP F127C has been meanwhile detected in a variety of different autoimmune diseases like psoriasis, Crohn's disease, Sjörgen's syndrome and RA, respectively, whereas the SNP rs6920220 has been shown to be a risk allele for RA and SLE only [18-22].

Type 1 diabetes (T1D) represents another autoimmune disease, where death of pancreatic β-islets is caused by the accumulation of inflammatory mediators in whose expression NF-κB and A20 as negative regulator of NF-κB are involved. A study of Fung et al. showed, that polymorphisms identified also in RA, SLE and Crohn's disease, could be also detected in T1D [23]. A list of different SNPs affecting A20 expression and/ or function in immune-related processes is depicted in Table 1. Noteworthy, several SNPs seem to be implicated in more than one autoimmune disease, while others might have protective or negative effects depending on disease type and population [21].

SNP	Location	Nucleotide	Position	Disease association	Population
rs13207033	223 kb upstream	A/ G	137965418	RA	American
rs13192841	221 kb upstream	A/ G	137967214	SLE	European
				Sjörgen's syndrom	European
rs2327832	215 kb upstream	A/ G	137973068	Coeliac Disease	European
				RA	European
rs10499194	186 kb upstream	C/ T	138002637	RA	European/ African-American, American, European
				Juvenile idiopathic arthritis	Japanese
				SLE/ RA	European
				Type 1 diabetes	European
rs6920220	182 kb upstream	A/ G	138006503	RA	American, European, European/ African-American

				Juvenile idiopathic arthritis	European
				Type 1 Diabetes	European
				SLE	European
rs7753394	103 kb upstream	C/ T	138085248	Crohn's disease	European
rs10499197	56 kb upstream	G/ T	138132516	SLE	European
rs5029930	Intron 1	A/ C	138190684	Coronary artery disease in Type 2 diabetes	American
rs5029937	Intron 2	G/ T	138195151	RA	European
rs5029939	Intron 2	C/ G	138195723	Systemic sclerosis	European
				SLE	European/ African-American
rs5029941	Exon 3	C/ T (A125V)	138196060	SLE/ IBD	African-American
rs2230926	Exon 3	T/ G (F127C)	138196066	SLE	European, African-American, Japanese, Chinese
				SLE/ RA	Japanese
				Sjörgen's syndrome/ Crohn's disease/ psoriasis/ RA	European
rs582757	Intron 5	A/ G	138197824	Rheumatic heart disease	Chinese
New (no rs)	Intron 5	C/ -	138197889	RA	European
rs610604	Intron 6	A/ C	138199417	Psoriasis	Caucasian
				Coronary artery diseases in Type 2 diabetes	American
rs5029953	Intron 7	A/ G	138200760	SLE	African-American
rs7749323	26 kb downstream	A/ G	138230389	SLE	European
rs5029930	28 kb downstream	A/ C	138232377	Coronary artery disease in Type 2 diabetes	American
Polymorphic dinucleotide	68 kb downstream	TT>A	138272732-138271733	SLE	European/ Korean
rs6922466	256 kb downstream	A/ G	138444930	SLE	European
				Sjörgen's syndrom	European

Table 1: Genetic variants of A20 related to autoimmune disease as adapted from Vereecke et al. and Nocturne et al. [11,12,22].

A20 and hematological neoplasms

A20 is known to act as a tumor suppressor in lymphoid malignancies. Aberrant NF-κB activation is implicated in tumor development and progression and recent studies have proven evidence that somatic mutations or epigenetic mechanisms affecting *A20* are involved in the development and progression of several lymphoid malignancies. Somatic mutations and/ or deletions as well as promotor methylation of *A20* lead to its inactivation and consequently to an over-activated NF-κB pathway in malignant cells with a reduced apoptotic potential due to an enhanced expression of anti-apoptotic genes. Importantly, accumulation of proliferating cells combined with the reduced ability to eliminate them, favors the augmentation of mutations within a given proliferating cell population and thus gives rise to further malignant somatic transformations. The mutations are not clustered into a specific region of the protein, rather they affect different regions ranging from the C-terminal OTU domain to the N-terminal Zn-Finger domains [10]. Likewise, the mutations observed can be varying, ranging from deletions, nonsense mutations introducing premature stop codons, missense mutations, frameshift mutations or insertions to nucleotide substitutions at splice donor sites. Several articles published within the last decade have highlighted the involvement of *A20* in non-Hodgkin and classical Hodgkin lymphomas as well as Burkitt's lymphoma, natural killer (NK) cell lymphoma, and adult T cell leukaemia (ATLL). Mono- and biallelic inactivation by deletions at 6q23.3 as well as different somatic mutations could be detected in mucosa-associated lymphoid tissue (MALT) lymphomas of the ocular adnexa, salivary gland and thyroid- but not of the lung, stomach or skin- , mantle cell lymphoma (MZL), activated B cell or germinal centre B cell like diffuse large B cell lymphomas (ABC-DLBCL and GCB-DLBCL, respectively), primary

mediastinal B cell lymphomas (PMBL), marginal zone lymphomas (MZL), follicular lymphomas and classical Hodgkin lymphomas (cHL) [24-33]. Interestingly, the occurrence of monoallelic deletions, having an inhibiting effect on A20 expression and function, indicates that A20 deletion might also act in a haplo-insufficient way and/ or in combination with gene amplification due to mutations in other proteins positively influencing NF-κB signalling like caspase recruitment domain-containing protein 11 (CARD11) [34]. Moreover, changes in A20 function have been attributed to epigenetic mechanisms like promotor methylation, which were particularly found in MCL, MALT lymphomas and lymphomas of the ABC- and GCB-DBCL subtype [26,29].

Recent work by our group could show that monoallelic deletions together with an increased expression of NF-κB target genes could be found in multiple myeloma (MM), another type of haematological malignancy [35]. Additionally we were able to detect a germline SNP rs143002189 in MM, which leads to a missense single base pair substitution - c.2364G>A- and predisposes to de development of DLBCL [36]. The latter could also be found in HL and benign hemangioblastomas [30,37].

Concluding Remarks

A20 is known to act as a tumor suppressor in many pathogenic processes ranging from auto-destructive and still difficult-to-treat autoimmune diseases to severe and prone-to-relapse haematological neoplasms. Several single nucleotide polymorphisms affecting A20 could be identified as disease-related in autoimmune-diseases like RA, SLE and malignancies affecting the homeostasis of the gut microflora. Both, somatic mutations and deletions as well as epigenetic mechanisms seem to be responsible for inactivation of *A20* in haematological neoplasms of non-Hodgkin and Hodgkin lymphomas. Nevertheless, the role of many germline single nucleotide polymorphisms still remain to be elucidated in haematological malignancies, a fact, which would certainly impact risk stratification and treatment options.

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