



Review

Positive selection on apoptosis related genes

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ABSTRACT

Apoptosis is a form of programmed cell death crucial for development, homeostasis, immunity, spermatogenesis, and prevention of cancer. Positive selection acting on mammalian apoptosis related genes targets protein interfaces that interact with pathogens and also elements of signaling complexes. Selection appears primarily to be driven by the immune/defense related function of these genes. Moreover, competitive interactions could be driving positive selection among sperm cells, as well as the need for protection against female anti-sperm immune responses. Trade-offs in fitness are expected out of these selective pressures, which could explain the involvement of these genes in various diseases, including cancer.

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1. Introduction

There is a delicate balance between cell death and cell survival. During development, cell proliferation acts in a balance with cell death to sculpt the organs of the embryo. Tumor cells, cells infected by external pathogens or cells that are irreversibly injured have to be efficiently removed by the organism with minimal damage. Furthermore, a normal healthy adult human produces around 10^{12} cells per day and cells that are in excess need to be eliminated. This is especially true for the immune system, since after the clonal expansion of antigen-specific lymphocytes produced after an immune response is triggered, controlled cell death is needed to restore normal cell levels in order to avoid lymphoid neoplasia and autoimmunity [1]. Autoimmunity is also tightly constrained by the elimination of auto-reactive immune cells [2].

The elimination of cells through a controlled set of biochemical processes called programmed cell death (PCD) usually occurs via apoptosis and is morphologically characterized by cell shrinkage and subsequent engulfment by macrophages, with no damage to the adjacent tissues. An uncontrolled PCD results in disease, such as neurodegenerative disorders, ischemic injury, cancer, autoimmune disorders and chronic infections.

There are various pathways involved in cell death (Fig. 1). Molecules such as interleukins (IL) and interferons (IFNs, involved in inflammatory responses) occur early in the signaling cascades [3,4]. The intrinsic (or mitochondrial) pathway is the most ancient one [5] and involves the release of molecules trapped in the mitochondrial intermembrane space leading to apoptosis through direct DNA fragmentation [6]. It can also activate the caspase cascade, which is central in many apoptotic pathways [6]. The extrinsic pathway involves the activation of death receptors on infected/damaged cells by cytokines produced by immune system cells [6]. This leads to the direct activation of the caspase cascade and also of the intrinsic pathway. Immune system cells also produce granzymes, powerful proteases that efficiently kill damaged cells after being injected into their cytoplasm, either by direct DNA fragmentation or activation the caspase cascade [7,8]. Furthermore, two transcription factors balance cell life and death: p53 responds to various types of cellular stress and DNA damage by initiating cell-cycle arrest or triggering apoptosis [9]; and nuclear factor kB (NF-kB) complexes are crucial for protection against apoptosis, not only in normal cells, but also in cancer cells and cells infected by intracellular pathogens [10,11].

Despite the fundamental role of apoptosis and the fact that the equilibrium between cell life and death is fragile, apoptosis related genes in humans tend to show strong evidence for positive Darwinian selection [12]. This means that mutations that change the amino acid sequence accumulate in these genes at a higher rate than it is predicted for a gene where substitutions among species

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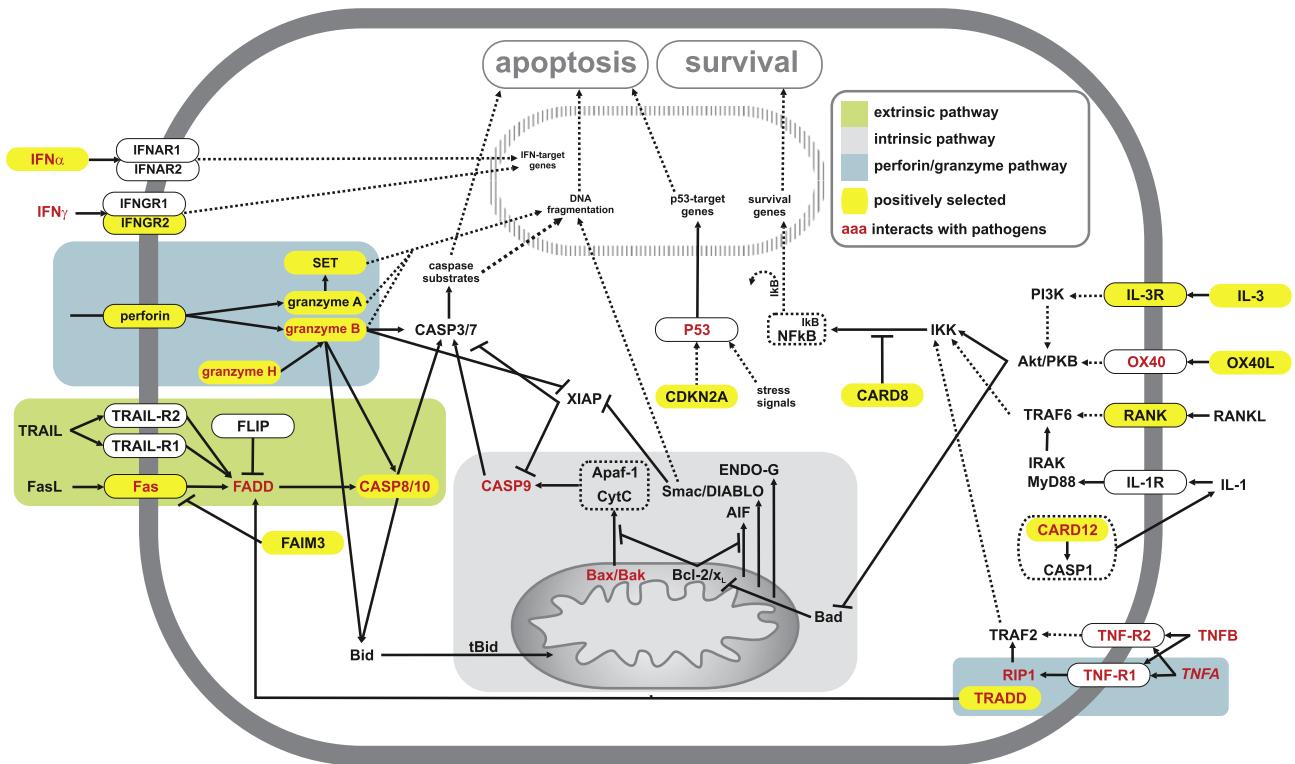


Fig. 1. The major pathways of cytotoxic T lymphocyte induced apoptosis and the mitochondrial intrinsic pathway. Representative steps of the NF- κ B pathway are also depicted. Molecules targeted by pathogens are indicated in red [45,64–69].

are driven only by mutation and subsequent genetic drift. In comparative data, positive selection acting on a coding sequence is generally detected by calculating the ratio of non-synonymous mutations (those that change the amino acid) per non-synonymous site to the number of synonymous mutations (that keep the amino acid) per synonymous site ($d_N/d_S = \omega$). If mutations are allowed to accumulate randomly (if there is no selection), ω is expected to be equal to 1. If the function of the gene is tightly maintained, and most mutations are neutral or deleterious, ω is expected to be below 1, indicative of negative selection (most genes, particularly housekeeping genes, fall into this category [13]). Positive selection ($\omega > 1$) occurs when there is some advantage in changing the gene function, such as in the case of proteins that recognize pathogenic molecules, and is characterized by rapid evolution. Importantly, positive selection is not indicative of functional importance in itself; positively selected genes may or may not be more functionally important than other genes. Rather, the presence of positive selection shows that the genes involved have provided evolutionarily adaptive responses to internal or external environmental changes, or otherwise have been involved in a dynamics that would selectively have favored changes of the genes. For more examples and an extensive description of positive selection at the molecular level see [14].

Given that apoptosis is critical to normal development and function, why are apoptosis related genes not highly functionally constrained, but seem to change faster than many other genes in mammals? A number of different hypotheses can be erected:

- (1) Positive selection may simply act to optimize protein function in a fixed environment. Many apoptosis related genes may be newly evolved genes that are still being optimized through natural selection, and are, therefore, still evolving rapidly.

(2) The selection acting on apoptosis related genes has to do with the role of apoptosis in immunity and defense. Many immune and defense related genes are evolving very rapidly to adapt to the constantly changing pathogenic environment [e.g. [15–19]].

(3) Positive selection could be driven by: (i) competitive interactions among sperm cells as suggested by Nielsen et al. [12], since selfish mutations contributing to the inhibition of apoptosis are favored by segregation distortion (meiotic products carrying such mutations may have a larger chance of developing into mature sperm cells); (ii) the need for protection against anti-sperm immune responses in the female reproductive tract.

However, given the pleiotropic nature of many of these genes, finding specific causes for selection on apoptosis related genes is difficult. The same apoptosis machinery is often involved in defense against pathogens, prevention of cancer, spermatogenesis, etc (e.g. see Tables 1 and 2 provided as Supplementary data). In addition, the pleiotropic effects of mutations in the genes are likely to cause trade-offs in fitness relating to different functions of the gene. Selection relating to, for example, viral defense may also affect other functions such as cancer prevention. However, mutations increasing functionality with respect to viral defense may not necessarily be optimal in terms of cancer prevention. Such trade-offs in fitness due to the multi-functionality of the apoptosis machinery may in itself help drive repeated positive selection, and help explain why these genes are not highly conserved despite their important functions.

A recent study has allowed further elucidation of the selective forces acting on apoptosis related genes. Kosiol et al. [20] performed a genome-wide scan in search for positively selected (PS) genes using six high-coverage genome assemblies for eutherian

Table 1Apoptosis related genes under positive selection as inferred by likelihood ratio tests implemented in PAML (P -value < 0.001).

Gene symbol	P -value	Function	Immune efficiency	Immune regulation and homeostasis	Cancer
EMP2	3.9E-09	Required for MHC1 expression			X
CASP10	8.3E-08	Proapoptotic (TNFR pathway)	X		
CARD8	1.7E-07	Proapoptotic (NF-kappaB pathway inhibitor)	X		
CCNB3	2.2E-06	Spermatogenesis (cell-cycle arrest)			
IFNA8	3.0E-06	Proapoptotic; antivirus	X		X
CTSG	3.1E-06	Proapoptotic; protease; inflammation	X		
CARD6	4.3E-06	Antiapoptotic	X		
HTRA4	5.6E-06	Proapoptotic; stress conditions			
GZMB	8.6E-06	Proapoptotic; antipathogen	X		
EMP1	9.4E-06	Expressed at high levels in proliferating cells	X		X
TNFRSF11A	1.4E-05	Proliferation (bone development)	X	X	X
CD3E	2.1E-05	T-Cell maturation		X	
CD3G	2.2E-05	Early thymic development		X	
CASP8	2.3E-05	Proapoptotic (TNFR pathway)	X		
GZMH	2.7E-05	Proapoptotic; antipathogen	X		
TMEM16J	4.5E-05	Proapoptotic; induced by p53			
HSPA1B	1.2E-04	Antiapoptotic under stress conditions			X
SET	3.3E-04	Antiapoptotic; GrA substrate		X	X
IL3RB	3.4E-04	Proliferation (hematopoietic cells); immunity	X	X	X
ARHGEF1	3.6E-04	Proapoptotic in thymocytes	X		X
COL4A3	3.6E-04	Proapoptotic in endothelial cells			X
NOX5	4.1E-04	Antiapoptotic	X		X
IFNA17	4.6E-04	Proapoptotic; antivirus	X		X
APOL6	5.5E-04	Proapoptotic			
CD5	6.1E-04	Modulates B- and T-cell receptor signaling			
SPN	6.3E-04	Proliferation and apoptosis in T-cells	X	X	
TNFAIP8L3	6.5E-04	Induced by tumour necrosis factor alpha			
PTPRC	8.3E-04	Signal transduction in T- and B-cells			
CDKN2A	9.7E-04	Apoptotic (indirectly activates P53); aging		X	X

mammals. From the ~16500 genes analyzed, Kosiol et al. identified 400 genes with strong evidence of positive selection ($FDR < 0.05$) and a larger set of 1596 with moderate evidence of positive selection (nominal $P < 0.05$). Ninety two of the latter were associated with apoptosis (Tables 1 and 2).

In this study we will provide a detailed discussion of the apoptosis related genes undergoing positive selection on the mammalian phylogeny based on the Kosiol et al. study and also on additional analyses of selected genes. We first present a summary of the apoptosis related genes under positive selection and we then focus on particular sets of genes grouped according to signaling pathways and/or biological and molecular function, in order to elucidate the factors causing positive selection in these genes. We will argue that genes involved in early signaling and that are very directly involved in early responses to pathogens are more likely to be under positive selection than other apoptosis related genes. We also argue that the timing of expression of various apoptosis related genes during spermatogenesis suggests a possible role for the third hypotheses outlined above. Finally, we make an overview of the relationship between apoptosis related PS genes and disease., and suggest that the rapid evolution of these genes may help explain the high prevalence of certain diseases. Our objective is not to review functional aspects of apoptosis, except when needed to understand the evolutionary hypotheses. We refer instead the reader to reviews in [1,6,7,21].

2. Methods

In this work we have used the 16529 genes selected as described in [20] and the set of positively selected genes that had nominal P values < 0.05 (total of 1596 genes). Statistical detection of positive selection can be sensitive to errors in sequencing, annotation and alignment [22]. Therefore, the set of orthologous genes proposed by Kosiol et al. [20] were subject to a series of rigorous filters for synteny, alignment quality, conservation of intron and exon structure and sequence quality [23]. Genes resulting from re-

cent duplication events were excluded from the analyses of positive selection (only high-confidence 1:1 orthologs were considered). Likelihood ratio tests (LRTs) were used to identify genes under positive selection ($\omega > 1$) by comparing two probabilistic models of variable ω ratios among sites, the simpler of which does not allow sites with $\omega > 1$ and a more general which does (see [20] for more information). In the following sections, P values for the test for positive selection will be presented within a parenthesis. GO [24] and PANTHER [25] ontologies were used to identify biologically meaningful categories. Apoptosis related genes have been defined as those that are annotated as such under either GO or PANTHER categories (total of 789). The Atlas of Genetics and Cytogenetics in Oncology and Haematology [26] was used to identify cancer-related genes (30% of the total number of genes in our sample). Fisher's exact test (FT) was used to identify an excess of PS genes in individual categories within the apoptosis- and cancer-related genes. The results were corrected for multiple testing using a FDR of 0.05. For a limited set of genes with available X-ray structures, we used the Bayesian empirical Bayes (BEB) method under the M8 model implemented in PAML [27] to determine the amino acid sites under positive (Fig. 3). For these analyses we used the alignments provided in Supplementary data that correspond to those in Ref. [20] plus extra available mammalian sequences for each gene. Three-dimensional protein structure models were built using Modeller [28]. The model for the extracellular domain of Fas complexed with FasL was built using the structures for TRAIL-R2 complexed with TRAIL and that of TNF-R1 complexed with TNFB as templates (PDB ids: 1DU3 and 1TNR, respectively). The interaction between the DD of Fas and the DD of FADD is represented as in the DISC structure (PDB id: 3EZQ).

3. Apoptosis related genes under selection

A list of some individual apoptosis related genes showing evidence of positive selection can be found in Table 1. There is a clear overlap among PS genes related to apoptosis, immune response,

cancer and other diseases (e.g. neuronal or cardiovascular diseases) (Fig. 2). We find that 48% of the PS apoptosis genes are involved in cancer, 12% in neuronal function/disorders, and 11% in cardiovascular diseases (Fig. 2). To some degree, these associations mirror those found in general among these categories, also in genes without positive selection.

Most of the apoptosis related genes under positive selection are related to the immune system ($P = 4E-02$), particularly in response to viruses ($P = 6.8E-03$) (see Table 3 provided as Supplementary data). In fact, viruses have developed numerous mechanisms for interfering with early antiviral signaling [29], which may explain the large number of genes under positive selection that participate in it.

4. Pathways under selection

4.1. Early rather than late (inflammation and cell signaling)

PS apoptosis related genes are located mainly upstream on the cascade events that lead to apoptosis/survival, at the level of cytokine activity ($P = 1.2E-02$) and transmembrane receptor activity ($P = 3.0E-02$). Clearly, selection acting to reduce or limit the effect of apoptosis would be expected to affect the very early stages of the apoptotic signaling and not the later stages. Positive selection relating to adaptation to apoptosis manipulation by pathogens leads to similar predictions: interference by pathogens on the host apoptosis machinery is much more efficient if it occurs before the cell already has begun the initial, typically irreversible, steps of the apoptosis process. Several signaling molecules and their receptors have been found to be under positive selection (Fig. 1). These are cytokines, such as interleukins (ILs) and interferons, which are molecules involved in intercellular signaling. These molecules are among the favorite targets of viral manipulation via the production of viral decoy receptors and proteins that sequester these cytokines [4,29].

4.2. The extrinsic pathway

We find that the genes in the extrinsic and granzyme pathways (Fig. 1) are significantly enriched with positive selection compared to those of the intrinsic pathway ($P = 3.1E-03$; Table 4 in Supplementary data). The intrinsic pathway is primarily involved in inducing apoptosis relating to internal stimuli such as cell damage. Signals resulting from the detection of foreign molecules, such as those from pathogens, are relayed by the extrinsic pathway. The excess of positive selection in the extrinsic rather than intrinsic pathway demonstrates that positive selection is not being driven by the role of apoptosis in processes relating to cell damage or other equilibrium processes (intrinsic pathway, Fig. 1), but is pri-

marily related to interactions with pathogens or other factors external to the cell. While the extrinsic pathway may be more recently evolved than the intrinsic pathway, it predates the chordate lineage [5,30]. Several genes are present outside the mammalian group. For example, the zebrafish harbors several elements of the death-receptor pathway [30], including Fas (Fig. 3A). It is therefore, unlikely that the excess of evidence for positive selection is caused by a recent origin of the genes in this pathway. This conclusion is also supported by the fact that positively selected sites present a number of amino acids with different chemical properties, indicative of repeated mutations (in Fas, for site 239, we find e.g. Val, Lys, Asp and Gln; for site 315, we find e.g. Leu, Trp, Gln and Met).

Others have previously observed an overall accelerated rate of protein sequence evolution in genes involved in caspase-dependent pathways [31]. The PS genes in the extrinsic and granzyme pathways are either directly (direct binding) or indirectly (homologue decoy pathogen-expressed molecules) affected by pathogens (see Fig. 1). The extrinsic pathway is important in immune and defense related apoptosis in response to viral infections, regulation of homeostasis of the immune system and is also thought to play an important role in apoptosis during spermatogenesis [32–35]. Apoptosis is triggered by the tumor necrosis factor ligands (e.g. Fas ligand (FasL)), which binds to tumor necrosis factor receptors (e.g. Fas, $P = 1.6E-03$, see Fig. 3A) [21].

The Fas/FasL system is essential in the control of the immune response homeostasis as shown by the uncontrolled accumulation of lymphocytes when it is affected by loss-of-function mutations [36]. FasL also acts as an immunosuppressant in immune privileged sites such as testis, eye, placenta and brain, and in tumors [37,38]. Fas receptors form homotrimers [39] that bind FasL molecules through their extracellular domains [40]. The intracellular death domain (DD) binds to Fas associated protein with death domain (FADD) which then binds the inactive pro-CASP8 ($P = 2.3E-05$) and pro-CASP10 ($P = 8.3E-08$), a step that precedes maturation of the caspases (removal of the pro-domain). c-FLIP can bind FADD and CASP8 inhibiting this step [41]. Mature CASP8/10 cleave CASP3/7, triggering cell death.

The positively selected sites in Fas fall into several regions crucial for signaling (see Fig. 3A). On the extracellular domain, the sites sit in the region that affects the pre-association of Fas molecules and ligand binding [39]. One positively selected site (239 in the human sequence) found on the intracellular DD sits on the interface with FADD, and changes from a small apolar valine in humans to a long positively charged lysine in the rat, which will very likely have a profound effect on the binding between the two molecules. Another PS site (315 in the human sequence) presents either a leucine residue (human) or tryptophan residue (rat). Curiously the side-chains of that site in two Fas molecules interact with each other, suggesting a major impact on the association between the different Fas molecules, essential to receptor activation [42].

What is driving positive selection in Fas? One possibility is the interaction with inhibitory viral molecules, since viral counterparts of c-FLIPs (v-FLIPs) and other viral molecules have been shown to interact with Fas, CASP8 and CASP10, inhibiting apoptosis [43–45]. Positive selection acting on Fas may result from the need to avoid inhibition by pathogenic molecules. Another possible contribution to the selective pressures on the extrinsic pathway elements is related to segregation distortion occurring in the male reproductive system, which will be discussed in the next section.

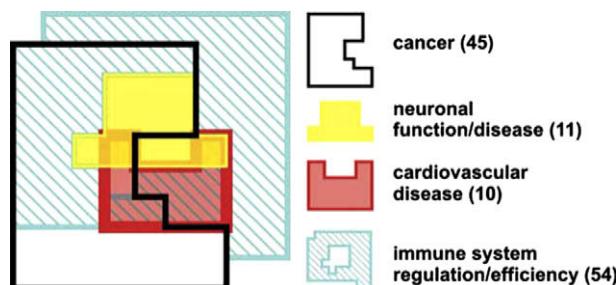


Fig. 2. Venn diagram representing the biological processes associated with the 92 positively selected genes involved in apoptosis found in our sample. The different elements are proportional to the number of genes for each category, indicated in parenthesis.

5. Spermatogenesis

Humans produce approx. 2×10^8 sperm cells per day, and up to 3/4 of the potential spermatozoa die by apoptosis in mammals [34]. This is thought to occur because of the limited capacity of

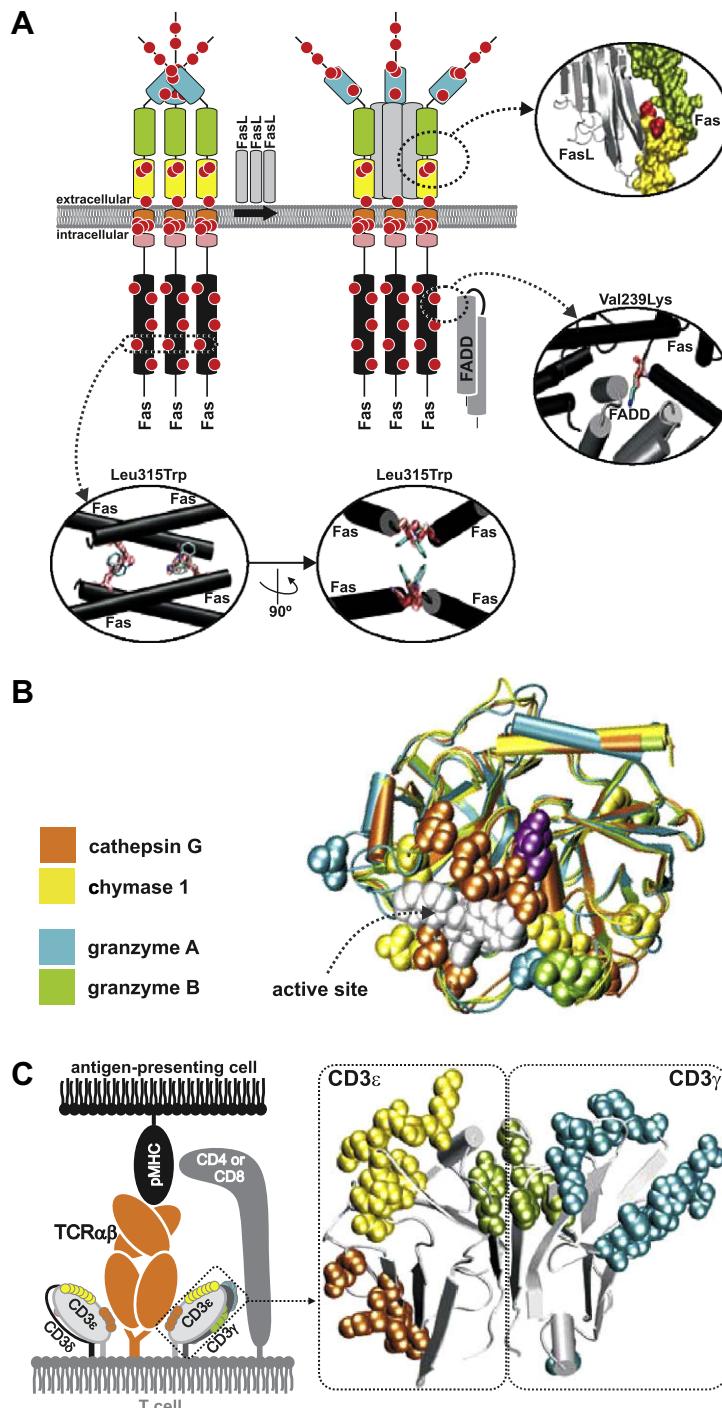


Fig. 3. Mapping of positively selected sites ($P > 0.9$) on protein structures of apoptosis related genes. (A) Fas trimer associated with FADD. Positively selected sites are shown in red. Side chains for residues 239 and 315 in humans (red transparent surface) and rat (sticks) are depicted for comparison. (B) Superposition of the crystallographic structures of four homologous serine proteinases implicated in apoptosis (PDB ids: CTSG (cathepsin G): 1T32; CMA1 (chymase): 1T31; GZMA (granzyme A): 1ORF; GZMB (granzyme B): 1IAU). Residues under positive selection are shown as spheres with colors according to the enzyme they belong to. The inhibitors bound to CTSG and CMA1 are shown as white spheres, depicting the active site. (C) Positively selected residues mapped on the CD3 ϵ heterodimer structure (PDB id: 1SY6). Residues in green indicate the interface between the two monomers, a region shown to be important for signal transduction [70]. Residues in orange are located around the suggested binding patch for the TCR $\alpha\beta$ receptor [71]. Antibodies to CD3 bind the area depicted in yellow [71,72]. Residues in blue cover a big extension of CD3 γ exposed surface, but have not been assigned any function yet.

the supporting Sertoli cells and/or to eliminate chromosomal abnormalities [46]. Apoptosis in adult male testis affects mainly spermatogonia, but also spermatocytes and spermatids, and a correlation has been found between the occurrence of apoptosis and the DNA replication phase in these cells [47,48].

Fas expression has been shown to be correlated with human germ cell degeneration in meiotic and post-meiotic arrest of spermatogenesis (Fig. 4) [35]. Furthermore, c-FLIP, an inhibitor of Fas-induced apoptosis (Fig. 1) has been shown to be expressed in spermatogonia, spermatocytes and spermatids [49], with a cell-specific

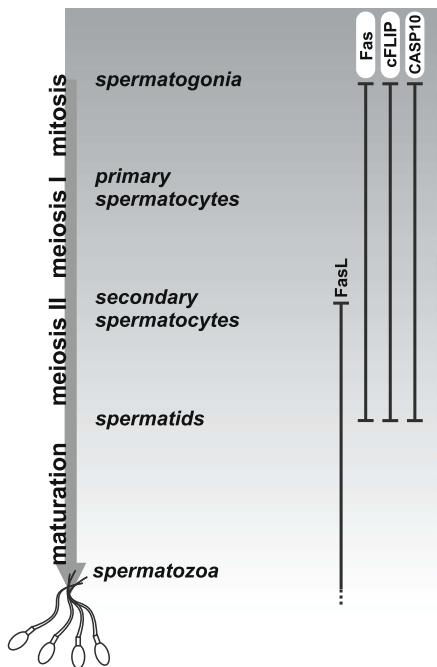


Fig. 4. Apoptosis related genes expressed in germ cells during the different stages of spermatogenesis.

expression pattern matching that of CASP10 [50] (Fig. 4). This indicates the need for protection against death-receptor pathway-mediated apoptosis during spermatogenesis.

The Fas/FasL system is suggested to be involved in at least two ways in the male reproductive system: (i) it eliminates defective and excessive cells during spermatogenesis; (ii) FasL protects spermatozoa against the immune response (e.g. lymphocytes displaying Fas) that takes place after insemination in the female genital tract [32,33]. Furthermore, the expression of FasL in sperm cells might also be involved in competitive interaction with other sperm cells as suggested in Nielsen et al. (2005). Interestingly, genes involved in Fas mediated apoptosis signaling within the cell tend not to be expressed after meiosis II. In contrast, FasL, capable of transmitting an apoptosis signal to other cells, is being expressed after meiosis II (Fig. 4). This is exactly as predicted if apoptosis is playing a role in competitive interactions among sperm cells, either from the same or from different individuals. However, the continued expression of FasL may also protect sperm cells against the female immune system. In either case, a competitive evolutionary dynamics will arise akin the host-pathogen interactions driving most of the positive selection in the human genome. It is likely that the role of apoptosis in spermatogenesis is an important factor driving positive selection relating to Fas mediated apoptosis.

6. Pattern recognition vs modulation of signal

Positive selection in immune defense-related genes is quite pervasive, in particular for genes specifically involved in the recognition of pathogen molecular patterns [15–19]. In the previous sections we have described how the direct contact with pathogens could be driving positive selection in a variety of apoptosis related genes. Another interesting example is that of a set of structurally related serine proteases responsible for cleaving protein substrates. Granzymes (see the perforin/granzyme pathway on Fig. 1), chymase, and cathepsin G are powerful enzymes all involved in immune defense. Fig. 3B displays four superimposed structures of these serine proteases, and we can see that, overall,

positive selection (sites shown as spheres) is targeting the loops that surround the active site area (occupied by inhibitor molecules in white). These loops have been shown to be responsible for substrate specificity and the control of activation of the enzymes. This leads us to suggest that the interaction with pathogens is driving positive selection in these enzymes, changing or broadening their specificity for foreign molecules, thereby calibrating their direct antimicrobial action and their role in the modulation of the immune response.

We have also found a number of apoptosis related positively selected genes that do not seem to be direct targets of pathogens, but are also involved in providing a healthy immune response. These genes are involved in either or both immune system development and efficacy, such as CD5 ($P = 6.1E-04$) that regulates both the selection process in thymocytes and the survival of activated B- and T-cells [51], or PTPRC (aka LCA or CD45, $P = 8.3E-04$) that is involved in both signal transduction through T- and B-cell receptors [52] and in early thymocyte development, taking part in the selection of CD4⁺CD8⁻ thymocytes, and B cell maturation [53]. Also the CD3 antigen, which is the signal transducing component of the $\alpha\beta$ T-cell receptor (TCR) complex in mature lymphocytes, is under positive selection (Fig. 3C). The $\alpha\beta$ TCR heterodimer is responsible for ligand recognition (MHC/peptide complexes) and subunits CD3 ϵ ($P = 2.11E-05$) and CD3 γ ($P = 2.19E-05$), which are components of the signal transduction system, have an essential roles in T-cell development [54,55]. The sites affected by positive selection seem to be involved in the efficiency in signal transduction (see Fig. 3C).

7. Cancer and autoimmune diseases

Cancer-related genes experience more positive selection than other genes (Table 3; $P = 1.9E-03$). This is largely explained by a strong association between PS genes and immune response (Table 3; $P = 4.1E-18$) and a general association between immune response genes and cancer-related genes (Table 3; $P = 2.9E-26$). The association between immune response and cancer, opens the possibility that much positive selection is responding to changes in trade-offs in fitness between the effects relating to the functional role of the genes in immune/defense, and the role of the genes in cancer. This will be especially important if the effects of changes in the gene on fitness, is opposite with respect to the two (or more) functions of the protein. A number of apoptosis related PS genes are involved in multiple processes associated with both tumor development and wound healing and inflammation, such as tissue invasion and metastasis (EMP1, $P = 9.4E-06$) and vascularization and angiogenesis (COL4A3, $P = 3.6E-4$) [56,57]. The same events involved in acute inflammation and healing also occur during carcinogenesis and chronic unresolved inflammation, although in a chaotic order [58]. We then might expect that changes that result in a better recovery from damage caused by infection could have a negative impact on fitness (e.g. a more efficient formation of blood vessels that rapidly restore oxygenation and provide nutrients to damaged tissues, may also contribute to better support of a developing tumor). Small changes in the pathogenic environment, may change the optimum of the trade-off of these pleiotropic effects of the gene, and induce it to evolve towards the new optimum (see Fig. 5).

A similar dynamic might arise due to the dual effect on changes in apoptosis related genes for defense against pathogens, and in autoimmune diseases. Indeed, genes involved in the immune response may at times have a negative impact on fitness. For example, genes involved in signaling in inflammatory processes play a major role in diseases such as rheumatoid arthritis [59]. Proteins with enzymatic activity, such as CTSG and CMA1, are also involved in autoimmune disease (e.g. asthma [60,61]). Both CMA1 and CTSG

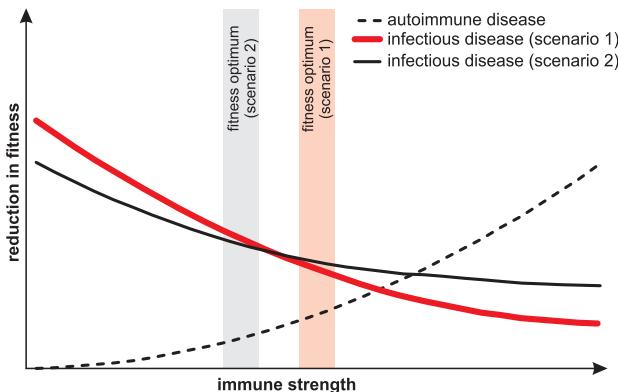


Fig. 5. Changes in the environment can lead to reduced fitness by altering the balance between an adequate immune response and disease.

are released by mast cells [61]. These are particularly abundant at the surfaces that interface with the external environment in the body, the main media for pathogen infiltration. Mast cells respond to danger signs of innate and acquired immunity by releasing several inflammatory mediators [62]. The immune hyper-responsiveness associated with CTSG and CMA1 might be the result of a dynamic that balances the strength of the immune response in a particular pathogenic environment, with the possible negative effects of hyper-responsiveness. Again, changes in the pathogenic environment may offset this balance and induce positive selection. For example, it is possible that, in recent human evolutionary history, as the density of the populations have increased, pathogens were more easily transmitted, leading to selection that might favor a different optimum in the trade-off between increased response to pathogens and decreased risk of autoimmunity disease. A trade-off between the collateral damage caused by the immunoresponse during infection and the efficacy of the response to pathogens may lead to a similar dynamic.

8. Conclusions

- (1) Death by apoptosis involves a very complex signaling system for eliminating excessive, defective or infected cells. It is one of the common targets of manipulation by pathogens, attempting to induce the death of immune system cells, or to delay the death of the cell they have colonized. As predicted under the immune/defense selection hypothesis, most positive selection appears to involve early signaling rather than late signaling, and does not appear to be related to intrinsic pathways involved in the elimination of defective cells. Instead, positive selection in a number of apoptosis related genes is likely driven by an evolutionary arms race between host and pathogens.
- (2) The expression pattern of apoptosis related genes under positive selection also lends support to hypotheses involving competition among sperm cells or interactions between sperm cells and cells from the female immune/defense system.
- (3) There are two main biochemical features that are changed by positive selection: (i) protein–protein interfaces interacting with molecules produced by pathogens (e.g. viral molecules such as v-FLIP); (ii) regions involved in the modulation of signaling (e.g. CD3).
- (4) Positive selection and antagonistic pleiotropic effects may help explain the segregation of apparently deleterious mutations relating to autoimmune disease, cancer, and other

apoptosis related functions. Many of the PS apoptosis related genes have multiple biological roles and so mutations in apoptosis related genes may, therefore, have antagonistic pleiotropic effects. This might in part explain patterns of repeated positive selection in apoptosis related genes, as compensatory mutations may follow initial beneficial mutations. For example, increased risk of cancer may be a negative outcome associated with positive selection related to the immune/defense, or other, functions of apoptosis genes. Mutations limiting apoptosis for certain germ cells, may provide a fitness advantage for the germ cell, but may lead to negative fitness effects for the mature organism in terms of increased risk of cancer or reduced response to pathogens. Likewise, mutations increasing the apoptosis response may lead to an improved response to pathogens, but an increased risk of autoimmune disease, etc. In changing environments, we would expect a changing balance in the trade-off between different effects of apoptosis to drive evolutionary changes at the molecular level. For example, the increased prevalence of autoimmune disorders has been linked to changes in human exposure to pathogens [63], indicating that adaptation to past environmental conditions leads to reduced fitness in the absence of those conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.12.022.

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