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Chapter 2

Apolipoprotein D

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Additional information is available at the end of the chapter

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Abstract

Apolipoprotein D (ApoD) is an extracellular glycoprotein of the lipocalin protein family, involved in different functions such as immune response, cell proliferation regulation, chemoreception, retinoid metabolism, axon growth, and proteolysis regulation. This lipocalin is expressed predominantly in the nervous system (NS), both prenatally (vascular pericytes) and postnatally (glia and neurons) and in adulthood. It is also expressed in other tissues and is carried by high-density lipoprotein (HDL) in plasma, so it could interfere in cholesterol and other lipids regulation. ApoD increases considerably in systemic apocrine gland tumors and also in some primary brain tumors. Although the specific biological role of ApoD is unknown, the presence of ApoD in tumors appears to be a prognostic factor in their evolution. Regarding the NS, increased ApoD expression observed in many neurodegenerative diseases could be used to make an early diagnosis thereof.

Keywords: oxidative stress, apolipoprotein, nervous system, colorectal cancer

1. Introduction

Apolipoprotein D (ApoD) is an extracellular glycoprotein of the lipocalin protein family, involved in different functions such as immune response, cell proliferation regulation, chemoreception, retinoid metabolism, axon growth, and proteolysis regulation [1, 2]. This lipocalin is expressed predominantly in the nervous system (NS), both prenatally (vascular pericytes) and postnatally (glia and neurons) and in adulthood [3, 4]. It is also expressed in other tissues and is carried by high-density lipoprotein (HDL) in plasma, so it could interfere in cholesterol and other lipid regulation [5].

ApoD increases considerably in systemic apocrine gland tumors and also in some primary brain tumors [6]. Although the specific biological role of ApoD is unknown, the presence of ApoD in tumors appears to be a prognostic factor in their evolution. Regarding the NS, increased ApoD expression observed in many neurodegenerative diseases could be used to make an early diagnosis thereof [6].

Under cellular stress conditions, ApoD presents extra- and intracellular overexpression [7, 8], suggesting that it plays a fundamental role in cell proliferation, survival, and death.

2. Structure and metabolism of ApoD

ApoD gene is located on human chromosome 3 and chromosome 16 in rodents. Its amino acid sequence does not maintain similarity to other apolipoproteins but is highly similar to some members of the lipocalins family [6, 9].

The molecular weight of mature human ApoD is 19 kDa. It consists of 169 amino acids with glycosylation sites at residues 45 and 178, corresponding to asparagine. Its molecular weight is 32 kDa calculated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and approximately 18% of them are carbohydrates. The glycosylation pattern of ApoD varies depending on the site; these values correspond to plasma ApoD, where carbohydrates are less complex and extensive and glycosylation is therefore smaller than ApoD in other body secretions and tissues [6].

Secondary structure has been proposed as a small β-barrel structure constituted by eight antiparallels β-leaves (**Figure 1B**). Within this framework, hydrophobic residues are situated in

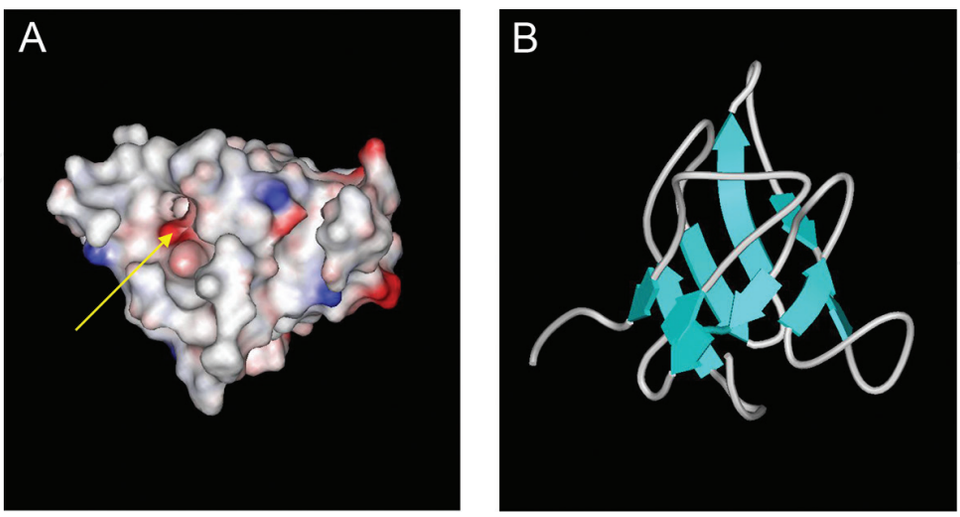

Figure 1. Tertiary structure of apolipoprotein D in three-dimensional vision. **A.** Model of protein surface. The pocket where hydrophobic ligands bind is indicated with an arrow. **B.** Model of α-carbon skeleton of the protein, also showing the eight β-chains that constitute the barrel of this lipocalin. Both figures have been set up with the program Weblab ViewerLite.

	Human	Rabbit	Guinea pig	Rat	Mouse	*E. coli*
Human	–	80%	78%	69%	71%	31%
Mouse	71%	82.6%	–	92.6%	–	–

Table 1. Similarity of the amino acid sequence in apolipoprotein D found in different species.

the inner surface of a central pocket (arrow in **Figure 1A**). This location would be the binding site for the ApoD ligand. Other hydrophobic residues could also participate in the association of ApoD with HDL particles [6].

It has been found both on a molecular as gene scale that ApoD is a heterogeneous protein. It has different isoforms and there is evidence of the presence of two alleles that are expressed in a codominant way in a single gene locus. The first fact can be explained, partly, by a posttranslational process, consisting sometimes in the addition of sialic acid. With respect to the gene, two different alleles were identified by digestion with enzymes Taq1 and Msp1.

Population studies show variations in ApoD gene as polymorphisms that may affect the function of ApoD, the lipoprotein metabolism, and plasma concentrations thereof. In fact, certain ApoD gene alleles show a significant correlation with the predisposition to certain neurodegenerative diseases [10].

The ApoD is also found in various mammals, with very similar functions to human ApoD, and whose similarity in amino acids is shown in **Table 1**. But it is found not only in mammals but also in birds with even greater similarity to human lipocalin than that of some mammals. There are homologous genes in insects and is even located in prokaryotes. In *Escherichia Coli*, there is a lipocalin (Blc) present in the outer membrane of the bacteria that maintain a 31% similarity with human ApoD. It is the first lipocalin located in bacteria and its expression occurs mainly during the stationary phase interacting with the response to hunger during this phase of bacterial cycle.

2.1. Location, synthesis, and expression of ApoD

ApoD has been detected in a variety of organs, tissues, and fluids, reflecting its importance and suggesting that it may play different roles depending on the organ in which it is located. It has been detected in plasma, tear fluid, in the eye ciliary body, in the cerebrospinal fluid (in concentrations without relation to plasma concentration), in the perilymph (in similar concentration to plasma), in the middle ear fluid, in urine, and in sweat [6].

Among the mammals, many interspecific differences in the ApoD expression can be found in different organs and tissues (see **Table 2**).

In contrast to other lipoproteins, the main synthesis of ApoD is not produced in the intestine and liver but in the adrenal glands, kidney, and central nervous system (CNS) [6]. Cells expressing as many ApoD mRNAs are perivascular fibroblasts, glial cells, pial and perivascular CNS, and some neurons [3, 4].

	Human	Monkey	Rabbit	Guinea pig	Rat	Mouse	Hamster
Brain	+	+	+	+	+	+	+
Liver	+	+	nd	+	+	+	−
Kidney	+	+	nd	+	+	-	−
Intestine	+	+	nd	nd	+	−	−
Pancreas	+	+	nd	nd	+	nd	nd
Placenta	+	nd	nd	nd	nd	nd	nd
Adrenal	+	nd	+	+	+	+	nd
Spleen	+	+	+	+	+	−	−
Heart	nd	nd	nd	nd	+	−	+
Bladder	nd	+	nd	nd	+	nd	nd
Skin	nd	+	nd	nd	+	nd	nd
Lung	nd	nd	+	+	+	+	nd
Testicles	nd	+	+	+	+	+	nd
Ovaries	nd	nd	+	+	−	+	nd

+: presence; −: Not present; nd: not determined.

Table 2. Differences in the expression of ApoD in different organs and tissues of several mammals.

2.2. Ligands and functions of ApoD

The ApoD is mainly part (83%) of the high density lipoproteins (HDL) [11], but can also be found in small amount in very low-density (VLDL) and low-density lipoproteins (LDLs) [6]. As a component of HDL, it was observed that ApoD is associated to cholesterol ester transferase protein (CETP), to Apo AI or Apo A-II (over 50% of ApoD present in HDL is forming part of these complexes with Apo A-II) [11]. In HDL, ApoD is also forming part of the complex responsible for the transport of cholesterol from peripheral tissues to the liver for its metabolism, especially with the lecithin-cholesterol acyltransferase (L-CAT). It is believed that ApoD could stabilize the enzymatic activity of the L-CAT or act as a substrate or reaction products carrier, such as cholesterol or cholesteryl esters, as an increase in the activity of cholesterol esterification by L-CAT in the presence of ApoD has been observed. All this suggests that interactions between cholesterol metabolism and ApoD exist, but there is evidence that cholesterol is not the main ligand of ApoD as initially believed. This is supported by the low affinity existing between them and by the fact that in the cyst fluid of breast cancer ApoD concentration increases up to 1000 times, while cholesterol increases only twice [6].

The ApoD can also be free or bound to other small molecules as it interacts with many ligands such as progesterone and other progestins, pregnenolone, bilirubin, arachidonic acid (AA), estrogens, androgens, and E-3-methyl-2-hexanoic acid (major component of underarm odor). Of all these, ApoD is the molecule that has a higher affinity for arachidonic acid, which make us believe that through the L-CAT, ApoD could join in the metabolism regulation, removing it

in order to prevent its transformation into cholesterol esters [6]. It is also important to mention that other linking molecule is retinoic acid, a molecule that plays a major role in the development of nervous system. Indeed, ApoD is the human lipocalin with higher affinity for retinoic acid [12].

Belonging to the family of lipocalin and the variety of tissues in which ApoD is expressed, Makes us pose the hypothesis that this apolipoprotein is multiligand and multifunction and that both function as ligands vary depending on the organ in which it is expressed [6].

2.3. Expression regulation of ApoD

Transcriptional regulation of the expression of ApoD seems to be very complex due to the many factors that modulate this protein [13]. Overall, we could say that there are changes in cell proliferation which modulate the expression of ApoD, or vice versa. Do Carmo et al. have studied in detail the ApoD promoter and determined the genomic region required for the induction of ApoD when cell senesces (when the crop exceeds the confluence). In this genomic region, there is a purine-pyrimidine fragment alternation and octanucleotide SRE (serum-responsive element) that appear to be essential for the induction. Curiously, SRE sites are present in sites related to cholesterol and fatty acids metabolism and mediate the regulation of transcription of these genes depending on sterol genes [13].

3. ApoD as a protective agent against oxidative stress

To conclude this section, we note that in studies conducted in animal models (particularly in the fruit fly *Drosophila melanogaster* and the mouse), in which the expression of ApoD has been genetically modified, ApoD deficit involves behavioral defects and neuronal death by apoptosis. Also, there is less resistance to NS stimuli that induce oxidative stress (OS). Moreover, lipocalin overexpression leads to increased resistance to factors which induce increased oxidative stress [14–16].

In summary, ApoD is a protein with multiple functions depending on the location in which it is expressed. It is a multiligand protein, but it does not mean that it has a specific role, so many studies are still needed to unravel the functional role of ApoD at different levels in which it operates. It has an important role in CNS pathologies, as it behaves as an acute phase protein, rising in neuronal damage. However, we cannot yet say that it acts as a neuroprotective or neurotoxic protein.

We also checked that oxidative stress induces the expression of ApoD in the nervous system [14]. This and the fact that many of the diseases mentioned above occur with increased oxidative stress made us think that this lipocalin plays an important role in controlling this stress when it occurs in pathological conditions.

Evidence from animal models supports this hypothesis. It is specifically carried out with experiments in *Drosophila* fly and mice. In the fly, the Glial Lazarillo protein (Glaz) is the homologous protein to ApoD. In Glaz mutant flies that inactivate its expression, subjected to

a stimulus that induces oxidative stress, neuronal death and degeneration are increased. By contrast, in flies with excess of ApoD there is a greater resistance to oxidative stress [16]. In experiments conducted in knockout ApoD mice (in which the gene is inactivated), we have found that these have also a lower resistance to oxidative stress and behavioral changes [17].

4. ApoD in nervous system and its relationship with neurodegenerative pathologies

We can find ApoD both in the central nervous system (CNS) and in the peripheral nervous system (PNS) and is part of the small group of apolipoproteins that are synthesized in the NS close to Apo E, J and C-I [6].

ApoD's role as a conveyor of lipid molecules suggests that it might play an important role in lipid transport during neuronal regeneration [6].

The ApoD accumulates in the peripheral nerve after its injury. Its concentration is much higher there than other apolipoproteins and also has been shown to be synthesized locally and not from the bloodstream, such as Apo A-I and Apo A-IV. In rat sciatic nerve injury, the concentration is increased 500-fold relative to baseline, and mRNA is elevated up to 40 times. Its mission might be to transport cholesterol to remyelination and new membrane formation. It could also carry bilirubin, which is found in damaged nerves, thereby preventing toxic accumulation of the same [6].

In adult animals, ApoD expression is primarily located in the pial and perivascular cells, astrocytes and oligodendrocytes and, inconsistently, in neurons [6].

ApoD has been observed attached to oligodendrocytes in the white matter of the human cerebral cortex. In the gray matter of young individuals, ApoD expression of both glial cells and neurons is limited; this expression increases with normal aging. It seems that ApoD synthesis could be linked to the phenomenon of cellular activation in astrocytes, taking place in astrogliosis. ApoD is constitutively secreted by mouse astrocyte cultures.

In neurons, labeling studies indicate that ApoD is more abundant in some areas than in others. In cerebrum and cerebellum, its labeling is poor and inconsistent, while in the vestibular nuclei, bulbar olive and raphe marking are abundant and constant.

In the nervous system, ApoD could participate in the process of regeneration and remyelination. It has also been proposed as lipids and other substances carrier through the blood-brain barrier. It could also play a significant role in maintaining appropriate levels of cholesterol in compartments not directly exposed to blood. Finally, as a function of this lipocalin the local transport of steroid hormones has also been proposed, which modulate the formation of synaptic connections [6].

4.1. ApoD and nervous injury

The relation between ApoD processes and nerve regeneration has conducted several studies in which injuries are reproduced in the CNS. In all of them, an increase in the ApoD marking has been observed, quite possibly of a local origin as ApoD concentration in the bloodstream

was low. This may be due to the need of increased lipid traffic during the period of reconstruction and restructuration following serious injuries. However, in a study conducted in deficient ApoE mice subjected to hypoxia To cause a stroke, no change was seen in the levels of ApoD with respect to wild strains, where this apolipoprotein would not be involved in cleaning lipid material in the area of the lesion and not be a part of the compensatory mechanism against the absence of ApoE.

4.2. ApoD and nervous pathologies

There are some nerve pathologies in which ApoD is elevated with respect to normal healthy individuals (see **Table 3**).

4.2.1. Niemann-Pick disease type C

In the mouse, this disease is a hereditary disorder of cholesterol homeostasis, which accumulates in unesterified form in the lysosomes. We found progressive dementia and development of neurofibrillary tangles. It was observed that the amount of ApoD in brains of diseased individuals was greater than that in healthy individuals and it was mainly secreted by astrocytes. This suggests ApoD intervention in cholesterol metabolism, acting as a conveyor of lipids released in demyelinating disease processes.

4.2.2. Alzheimer's disease

This disease is a disease of pathology substrate constituent of senile plaques and neurofibrillary tangles. These lesions are clinically reflected in a consistent mental decline in dementia, disorientation, memory loss, and learning capacity.

In these patients, increased ApoD is found in the hippocampus and CSF compared with control subjects. Also, a correlation was found between ApoD and the presence or absence of the ApoE4 allele, so that high concentrations of ApoD were interpreted as a compensatory mechanism against the absence of a particular allele of ApoE in nerve regeneration and maintenance and CNS repair. The ApoD would act in transporting different substances. This theory has been challenged by studies with ApoE-deficient mice in which the expression of ApoD was not altered by the absence of ApoE.

Other studies indicate an ApoD increase in the entorhinal and temporal cortex in elderly subjects. According to some authors, ApoD could participate in the neurochemical cascade associated with chronic CNS neuronal degeneration. Other studies indicate that there is no correlation between ApoE and ApoD and both are involved in neurodegeneration in this disease independently.

Moreover, the analysis of senile plaques and neurofibrillary tangles has resulted in conflicting data, some authors find ApoD presence while in other cases not. This may be due to the employed antibodies. In case of ApoD found in senile plaques, ApoD suggests an important role in fibrillogenesis and deposition of amyloid peptide. The presence of ApoD in neurofibrillary tangles is little or null although we have found a correlation between the number of tangles and the amount of ApoD in patients. It has been interpreted as the injured cortical neurons increase the expression of ApoD before tangles accumulate inside. The concentration of

Pathology/alteration nervous system	Overexpression site	Detected Increase		Ref.
		mRNA	Protein	
Cerebellar ataxia (two mouse models)	- Cerebellum	+		[18]
Unverricht-Lundborg disease (Mouse model: progressive ataxia)	- Cerebellum	+		[19]
Niemann-Pick disease – type C (mouse model: progressive neurodegeneration, ataxia)	- Cerebellum, fraction myelinated - Globus pallidus, thalamus, substantia nigra. White matter in the internal capsule and cerebellum. oligodendrocyte precursors - Brain	+	+ +	[20] [21] [22]
Alzheimer's disease	- Cerebral spinal fluid - Pyramidal neurons with granulovacuolar degeneration - Cortex with neurofibrillary changes - Hippocampus - Deposits β-amyloid	+	+ + + + +	[23] [24] [25] [23, 26] [27]
Schizophrenia and/or bipolar disorder	- Blood serum - Caudate and Brodmann area 9		+ +	[7, 28] [7]
CNS demyelinating diseases (multiple sclerosis)	- Spinal fluid and serum (Intrathecal production)		+	[29]
Astrocytomas (pilocytic and other noninfiltrating)	- Astrocytoma	+	+	[30]
Transmissible spongiform encephalopathies (Mouse)	- Brain	+		[31]
lethal Sindbis virus encephalitis (mouse)	- Central nervous system	+		[32]
Response to neuroleptic drugs (mouse)	- Fluted, globus pallidus, thalamus, and white matter	+	+	[7, 28, 33]
Neuropathic pain after spinal nerve ligation L5 and L6 (rat)	- Dorsal root ganglion	+		[32]
SNP damage (rat): - section sciatic nerve	- site of injury - Fibroblasts in the perineural space	+	+	[34] [35]
CNS damage (rats): - Entorhinal cortex injury - Kainic acid in hippocampal CA layer	- Ipsilateral Hippocampus - Pyramidal neurons in the lesion	+	+ +	[36] [37]
Aging under normal conditions	- reactive astrocytes in cerebral cortex	+	+	[24, 38, 39]

Table 3. Pathological situations or cell damage which overexpresses the ApoD gene and/or protein accumulates in the SN.

this protein in the hippocampus appears to be related to the severity of intraneuronal neurofibrillary changes, but not with extracellular amyloid peptide level. Thus, the most advanced patients (according to the scale of Braak) have a higher content of ApoD.

ApoD has also been located in the vascular preamyloid and amyloid deposits of cerebral amyloid angiopathy, present in most Alzheimer's patients and which is common in the elderly. These patients are at an increased risk of vascular rupture. An inverse behavior has been

shown between ApoD and ApoE which would indicate that both proteins have different roles in the development of the disease.

It has also been shown the existence of an increased ApoD expression in rats expressing the mutated protein of human amyloid peptide precursor (characteristic of this disease). These changes were most striking in the hippocampus fimbria, corpus callosum, and other white matter tracts. This may represent a compensatory glial response to amyloid peptide deposition in Alzheimer.

4.2.3. Spongiform encephalopathies

An increase in ApoD expression has been shown, especially in later stages, possibly as a result of cellular stress.

4.2.4. Demyelinating diseases

In all of them, the elevation of ApoD is found in CSF, possibly due to rupture of the blood-brain barrier, but showed an inconstant behavior in relation to the plasma protein levels. In multiple sclerosis, there is also an increase in ApoD intrathecal production in the early stages of the disease. This has been considered a consequence of demyelination and remyelination processes that characterize the disease in the early stages. Also, increased ApoD has been described in patients treated with steroid.

4.2.5. Schizophrenia and bipolar disorder

In these psychiatric disorders, the expression of ApoD also increases in serum and brain, acting as disease marker. In schizophrenia and schizoaffective disorders, a decrease of arachidonic acid is found in the membrane of blood cells, fibroblasts, and brain tissues. We also found calcium-independent phospholipase A2 increased activity in psychiatric illnesses as a decrease of calcium-dependent phospholipase A2 activity [7]. It has shown an increase in the expression of ApoD in the striatum of rodents treated with clozapine and in various regions of white matter. In control animals, ApoD is mainly expressed in astrocytes while in treated animals the increase occurred mainly in neurons. This indicates a contribution of ApoD to antipsychotic mechanisms of this neuroleptic [6]. In addition, arachidonic acid also increases with treatment [7].

There are regional differences in ApoD expression when comparing schizophrenia with bipolar disorder, whereby ApoD could intervene in a natural response to the targeted effects of this neuropathology. In treated patients with schizophrenia, ApoD levels descend compared to normal values. The low concentration of ApoD in serum confirms the association of ApoD with a systemic deficiency in lipid metabolism. Serum ApoD levels of patients with schizophrenia are greater than those of control subjects, which may indicate the onset of the disease. Arachidonic acid (AA) is the precursor of eicosanoid synthesis and prostaglandin metabolism and relates to the formation of the second messenger cAMP. ApoD could link to transport and union of AA, prevent peroxidation and make it accessible for the synthesis of membrane phospholipids, protecting the neuronal membrane functions [7].

Finally, we should note that in studies carried out in animal models (particularly, in the *D. melanogaster* fly and mouse), in which ApoD expression has been genetically modified,

the deficit of ApoD involves behavioral defects and neuronal death by apoptosis. Also, there is less resistance to SN stimuli that induce oxidative stress. Moreover, lipocalin overexpression leads to increased resistance to factors which induce increased oxidative stress [9, 15, 16].

In summary, ApoD is a protein with multiple functions depending on the location in which it is expressed. The fact that it is also multiligand does not allow us to point a specific role for this protein; so, many studies are still needed to unravel the functional role of ApoD at different levels in which it operates. What we can say is that it has an important role in CNS pathologies. We cannot yet determine if it acts as a neuroprotective or neurotoxic protein, but we can affirm that it behaves as an acute phase protein, rising in neuronal damage.

4.2.6. ApoD gene polymorphisms

As mentioned earlier, we have determined four gene polymorphisms ApoD in the patients in our study.

Polymorphism rs1467282, c.334 + 718T > C in intron 4 of the gene was determined as genetic variation ApoD in Alzheimer's disease in Finnish population [10]. The theoretical European population frequencies given in **Table 4** are as NCBI database—single nucleotide polymorphism (http://www.ncbi.nlm.nih.gov/projects/SNP).

The polymorphism rs 5952 c.44T> C showed increase risk of sporadic Alzheimer's disease in Chinese population. Rs rs 1568566 haplotype T 5952 C showed lower risk and could be interpreted as a protective factor against Alzheimer's disease [40]. No data frequency in European population was seen.

With respect to Rs1568565 polymorphism c.124-352A > G (intron 2), the -352G allele was associated with a threefold increase risk of Alzheimer's disease early onset (≤65 years) Finnish population [10]. The theoretical European population frequencies given in **Table 5** are as NCBI database—single nucleotide polymorphism (http://www.ncbi.nlm.nih.gov/projects/SNP):

As we see, ApoD could confer protection against damage in the body by oxidative stress, certain polymorphisms ApoD could act in these diseases protecting the carriers of said damage. That is why we think that variations of this gene may confer susceptibility to damage caused by oxidative stress.

Genotype/allele	CC	CT	TT	C	T
Frequency	0.860	0.116	0.023	0.919	0.081

Table 4. Data of theoretical frequencies of rs1467282 in European population.

Genotype/allele	CC	CT	TT	C	T
Frequency	0.833	0.125	0.042	0.896	0.104

Table 5. Data of theoretical frequencies of rs1568565 in European population.

5. Vascular actions of ApoD

As mentioned above, the 1–2% of HDL is formed by ApoD. Likewise, we can say that 83% of plasmatic ApoD is found forming part of HDL particles. Thus, it has been found that decreasing ApoD in HDL before it descends in serum increases the risk of stroke. The fact that the HDLs are responsible for transporting cholesterol from peripheral tissues to the liver and that most ApoDs are present in these lipoproteins makes us think about its beneficial effects from the cardiovascular standpoint [11].

The presence of ApoD has been detected in the atheromatous plaque, but not in normal coronary arteries [11]. An important finding is the large amount of ApoD in quiescent cells [13]. However, its expression is greatly reduced in proliferating cells. In vitro studies show that ApoD inhibits cell proliferation, obtaining a similar effect to that produced by calcium antagonists, demonstrating its beneficial effects in the cardiovascular field. Also in his role of inhibiting proliferation could play an important role in cancer [11].

It has been observed that ApoD is related to the cell migration of vascular smooth muscle for closing the ductus arteriosus and the platelet-derived growth factor—BB (PDGF-BB)—may mediate its expression and localization. It has also been found that ApoD is necessary for the migration of pulmonary artery in response to PDGF-BB.

6. ApoD and tumor pathology

ApoD is associated with reduced proliferative activity of cancer cells, and is abundantly raised in senescent cells. In breast cancer, ApoD expression is associated with favorable histology and clinical stage, whereas in adjacent tumor stromal ApoD expression is a marker of adverse prognosis. Estrogen receptor expression in breast cancer is inversely related to ApoD expression. Therefore, a combined estrogen receptor positivity/ApoD positivity could reflect a nonfunctional estrogen receptor pathway, and this subset of breast cancer patients does not react to adjuvant tamoxifen treatment [41].

Our group has conducted studies in the field of colorectal cancer (CRC) and ApoD. In CRC, tumor growth coincides with increased inflammation, COX-2, and nuclear factor kappa B-mediated, which triggers the release of tumor necrosis factor alpha and interleukin-6 [42]. This inflammation increment corresponds to increased levels of reactive oxygen species (ROS) and their reactive derivatives, inducing in turn oxidative stress (OS) [43–45]. CRC cells show an increase in lipid peroxidation by products that could be triggered by the increased arachidonic acid levels attained by the increased activity of COX-2 [46, 47]. The accumulation of lipid peroxidation results in cell damage and death. However, cancer cells tend to reduce the levels of the ant proliferative cytokine TGF-β1 and the lipid peroxidation adduct 4-hydroxynonenal (4-HNE) as a way to prevent apoptosis.

We have seen that ApoD is related to protection against oxidative stress. It has also been linked to decreased cell proliferation in models [11, 48]. Its expression is regulated by p73 and p63, both p53 family members, which is tumor suppressor so that its expression shows an inverse correlation with tumor growth [49].

As we can see, ApoD levels are increased in the oxidative stress as a defense mechanism but in turn are reduced in advanced stages of cancer. Our group has studied mechanisms that contribute to this paradox and the influence of ApoD in cancer progression and patient survival.

Our results show a repression of ApoD gene expression in CRC, particularly in the initial stages of the disease, which correlates with an elevation of lipid peroxide, adducts in the tissue. In normal mucosa, ApoD protein is present in *lamina propria* and enteroendocrine cells. In CRC, ApoD expression is heterogeneous, with low expression in stromal cells commonly associated with high expression in the dysplastic epithelium. ApoD promoter is basally methylated in HT-29 cells but retains the ability to respond to OS. Exogenous addition of ApoD to HT-29 cells does not modify proliferation or apoptosis levels in control conditions, but it promotes apoptosis upon paraquat-induced oxidative stress [50].

Our results show ApoD as a gene responding to oxidative stress in the tumor microenvironment. Besides using ApoD as marker of initial stages of tumor progression, it can become a therapeutic tool promoting death of proliferating tumor cells suffering oxidative stress [50].

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