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Review

Emerging concepts of apolipoprotein D with possible implications for breast cancer

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Abstract. Apolipoprotein D (ApoD) is a small glycoprotein of 24 kD, and a member of the lipocalin family. ApoD exerts several intracellular mechanistic roles, especially ligand binding. Some putative ligands are arachidonic acid, progesterone, and tamoxifen. It probably has a binding/reservoir function of these ligands in the cytoplasm. Furthermore, ApoD has features compatible with endosomal trafficking, proteolytic activity and interactions in cellular signal pathways. ApoD inhibits translocation of phosphorylated MAPK into the nucleus. Moreover, 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 favourable histology and clinical stage, whereas in adjacent tumour stroma ApoD expression is a marker of adverse prognosis. Oestrogen receptor expression in breast cancer is inversely related to ApoD expression. Therefore, a combined oestrogen receptor positivity/ApoD positivity, could reflect a non-functional oestrogen receptor pathway, and this subset of breast cancer patients does not react to adjuvant tamoxifen treatment. The triangular relationship between oestrogen receptor, tamoxifen and ApoD should be further explored.

Keywords: Apolipoprotein D, breast cancer, molecular, biology, prognosis, predictive factor, tamoxifen, review

1. Introduction

Apolipoprotein D (ApoD) a small glycoprotein of 24 kD, a member of the lipocaline family, is expressed in most body tissues and has important roles in normal human cell physiology. Pathophysiologically, it is associated with defects in lipid metabolism and cancer cell progression. ApoD was first detected in 1963 in human plasma [2]. Ten years later a progesterone-binding component in cyst fluid from women with gross cystic disease of the breast (i.e. fibrocystic disease of the breast) was identified that differed from the sex-hormone binding properties of serum [72]. In 1977, Haagensen et al. [31,32] characterized and named this protein gross cystic disease fluid protein-24 (GCDFP-24). Eventually, others named it progesterone-binding cyst protein (PBCP) [49,92]. In 1990, Balbin et al. [4] examined the molecular structure of GCDFP-24 (i.e. PBCP) and found it to be identical to ApoD.

Despite its interesting biochemistry and putative functions, ApoD is relatively unknown. Two explanations are obvious: First, various articles on ApoD are spread over widely different journals. Secondly, its location and function is confusing: ApoD can be located in the cytoplasm [82,100,110], near the outer cell membrane [100], and in the perinuclear membrane area [81,100] (Fig. 1). Due to these different cellular locations, its function is more complex to understand than that of proteins with a subcellular localization mainly in the nucleus [59] (e.g. oestrogen receptor (ER), Ki 67). This difficulty may have contributed to the relative paucity of ApoD studies.

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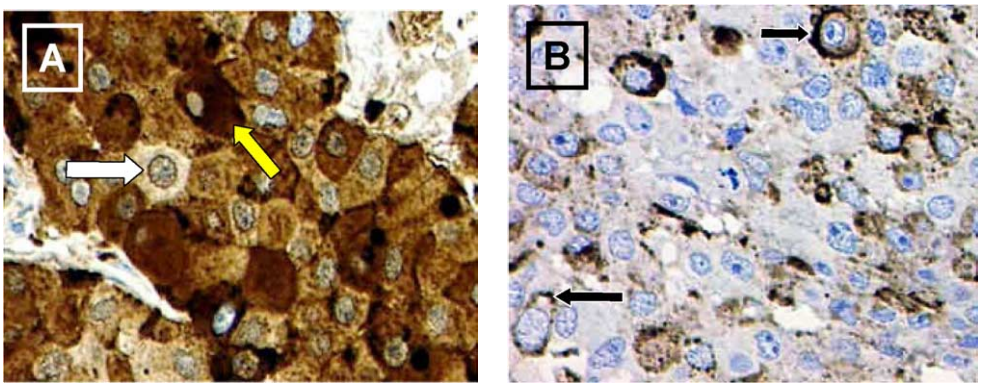
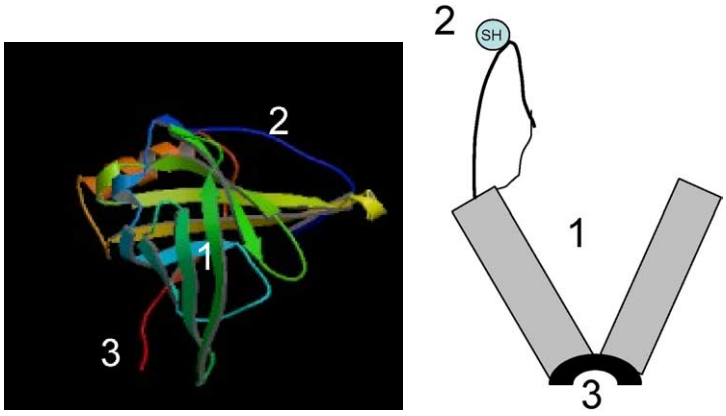


Fig. 1. Immunohistochemical determination of ApoD in breast cancer tissue. (A) Strong cytoplasmic (yellow arrow) and perinuclear membrane area (white arrow) staining. (B) Staining of outer cell membrane (black arrows).



(A) (B)

Fig. 2. (A) Ribbon model [66] of the ApoD molecule with the three main structure/functional features (1, 2, and 3 in the figure): 1 – The bands in green (4 bands), blue (2 bands), and yellow (2 bands) form the anti-parallel β-barrel structure of the ligand-binding cup where hydrophobic molecules are bound. 2 – Ω-loop containing cysteine-rich residues with one free hydrogen–sulphur group (SH) that may bind to other molecules with cysteine residues and form dimers and trimers and complexes with other molecules. 3 – N-terminal 3_{10}-α-helix found in proteins associated with endosomal trafficking serving as a docking device on intracellular membranes. (B) Cartoon model of ApoD with the same three main features as shown in (A).

In this paper, we would like to focus on ApoD and its interactions by addressing contemporary molecular aspects of possible relevance for its biological understanding. In particular, we want to deal with the relevance of ApoD in breast cancer development and management.

2. Mechanistic aspects of Apolipoprotein D (ApoD)

The human *Apolipoprotein D* (ApoD) gene is located on chromosome 3q26.2-qter and contains 5 exons [20,65]. The promoter region, located 10 kbp upstream from exon 1 [42], has response elements for oestrogen, glucocorticoids, progesterone, vitamins A and D and many other molecules [19]. The *ApoD*-gene product is a small glycoprotein of 24 kD, consisting of 169 amino-acids (Fig. 2). Based on homology regions, ApoD is classified in the lipocalin family, is found in plasma, and is also expressed intra-cellularly. Despite sharing homology with other lipocalins with a known membrane receptor (megacalin receptor), no receptor for ApoD has been classified as yet [23]. Unlike other apolipoproteins, which are primarily expressed in the intestine and liver, ApoD is more abundant in many other tissues, including adrenal glands, breast, endometrium, placenta, brain, and connective tissue [8,20,66,90].

The widespread location of ApoD suggests an important functional role in human cell physiology. In spite of its small size, the 3-dimensional structure has three main features [25,66] (Fig. 2):

1. The *8 anti-parallel β-barrel* structure of the ligand-binding cup where hydrophobic molecules are bound;
2. An Ω-*loop* containing cysteine-rich residues with one free hydrogen–sulphur group (SH) that may bind to other molecules with cysteine residues and form dimers and trimers and complexes with other molecules;
3. An *N-terminal* 3_{10}-α-*helix* found in proteins associated with endosomal trafficking [69] serving as a docking device on intracellular membranes. (For more biochemical details of ApoD reference is made to [25,75].)

ApoD takes part in complex interactions with a number of target molecules playing a central role in important pathways in the regulation of cell function; it has features compatible with transport proteins of hydrophobic ligands [25,69], epitopes with receptor binding properties [23], formation of homologous and heterologous oligomers, and proteolytic activity [47]. *In vitro* studies have shown ApoD to be a tissue-specific multi-functional hydrophobic ligand carrier/interacter with a strong binding affinity to arachidonic acid [61], pregnenolone, progesterone [48] and bilirubin [27]. Moreover, tamoxifen has about 40% of the affinity of progesterone to ApoD, while oestrogen have a very low affinity to ApoD [48]. After binding the ligand, ApoD can make a dimer/oligomere and the binding process is very fast [71]. This indicates that small changes in its target molecules (ligands) can be corrected or controlled very quickly by ApoD (e.g. recycling of free arachidonic acid back to cell membrane).

3. Regulation of ApoD

Based on pertinent literature [6,7,16,19,33,35,53–55,85–87,96], the transcriptional up- and down-regulation of ApoD expression mechanisms are briefly summarized in Fig. 3. Since there are three oestrogen receptor responsive elements in the promoter region, the transcription of ApoD is very sensitive for a functional ER–oestradiol complex. At the epigenetic level, hypermethylation of CpG islands in the promoter region is an effective way to down-regulate transcription of a gene [19]. In non-endocrine malignant tumours, including colon cancer [64], hepatocellular carcinoma [101], and oesophageal cancer [108], heavy methylation in the promoter region of the ApoD gene has been shown, and subsequently the expression of ApoD was found to be down-regulated.

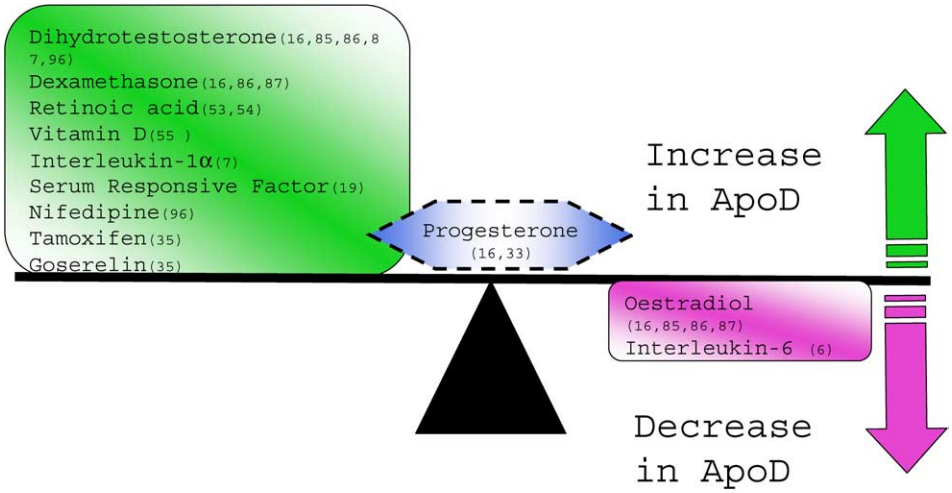


Fig. 3. Regulation of ApoD transcription. Factors on the left side (indicated in green) increase transcription. Factors on the right side (indicated in purple) decrease transcription. In the middle (in blue) is progesterone, which may both increase and decrease ApoD expression and sometimes has no influence at all on transcription. Figures in brackets correspond to the reference list. Of note are the well-documented stimulating effect of androgens and glucocorticoids and the inhibiting effect of oestradiol. The mechanism of tamoxifen and goselerin in increasing ApoD transcription is through inhibition of oestradiol. (Oestradiol has an inhibitory effect on the transcription.)

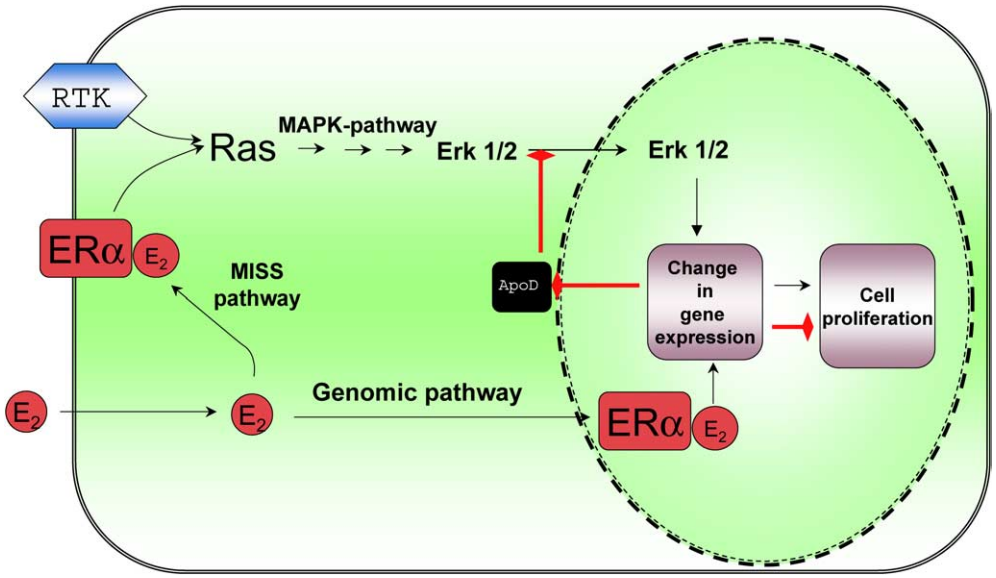


Fig. 4. ApoD and interaction in the MAPK and genomic –ERα and MISS–ERα pathways. In the MAPK pathway, ApoD is shown to inhibit (⟞) the translocation of Erk 1/2 (= MAPK) from the cytoplasm into the nucleus. Hence, transcription of factors that increase proliferation is inhibited. ERα stimulates proliferation (→), but inhibits (⟞) ApoD transcription. This inhibition of ApoD is "a gate opener" for the MISS-ERα pathway which acts through the Ras/MAPK pathway; consequently cell proliferation is enhanced. Abbreviations: ApoD: apolipoprotein D; ERα: oestrogen receptor α; E_2: oestradiol; Erk 1/2: extracellular regulating kinase; MAPK: mitogen activated protein kinase; MISS: membrane initiated steroid signalling; Ras: rat sarcoma virus oncogene; RTK: receptor tyrosine kinase.

4. ApoD interacts with the mitogen-activated protein kinase (MAPK) signalling pathway

In the mitogen-activated protein kinase (MAPK) signalling pathway, translocation of the phosphorylated activated form of Erk1/2=MAPK from the cytosol into the nucleus is required for cell cycle entry (Fig. 4) [11]. ApoD inhibits this translocation in ovine vascular smooth muscle cells [81], blocking cells from entering G_1 and thus inhibiting proliferation. Whether this inhibition results from suppression of the nuclear export proteins, activation of Rac or protein kinase A, or from blocking the binding of phosphorylated MAPK to the docking molecule inactive mitogen kinase phosphatase is still under investigation [81]. Interestingly, hyper-expression of MAPK mRNA has been observed in human breast cancer, including epithelial cells in the invasive front [89]. This finding is in agreement with the frequent observation that increased mitotic activity in the periphery of breast cancers is prognostically a strong sign [3]. Moreover, low activity of MAPK [63] and low proliferation activity [3] have been found to correlate with better relapse-free and overall survival in breast cancer. ApoD expression in cellular senescence could be explained by its ability to "turn off" the mitotic drive through inhibition of the important MAPK pathway, maintaining the quiescent state.

5. ApoD interacts with arachidonic acid and prostaglandin synthesis

ApoD has a high binding affinity for arachidonic acid [61]. Arachidonic acid is the substrate for the important prostaglandin-producing cyclo-oxygenase-2 (COX-2) and 5-lipoxygenase (5-LO) enzymatic pathways, which have prostaglandins and leucotriens as the end products. These end products stimulate cell proliferation, inhibit apoptosis, facilitate neo-angiogenesis (Fig. 5) and therefore are of the utmost importance for malignant transformation [58,78,97]. In short, (too much) free arachidonic acid is toxic for normal cells [15,73]. ApoD sequesters free arachidonic acid in the cytoplasm [100] and may stabilize membrane-bound arachidonic acid [99]. Hence, ApoD may be part of the Lands cycle where arachidonic acid is cycling between a free and an esterified form [44,73]. Both effects are thought to be of importance for the enzymatic conversion of arachidonic acid in the cell. ApoD therefore could play a role in regulating the amount of cyto-active factors in the COX-2 and 5-LO path-

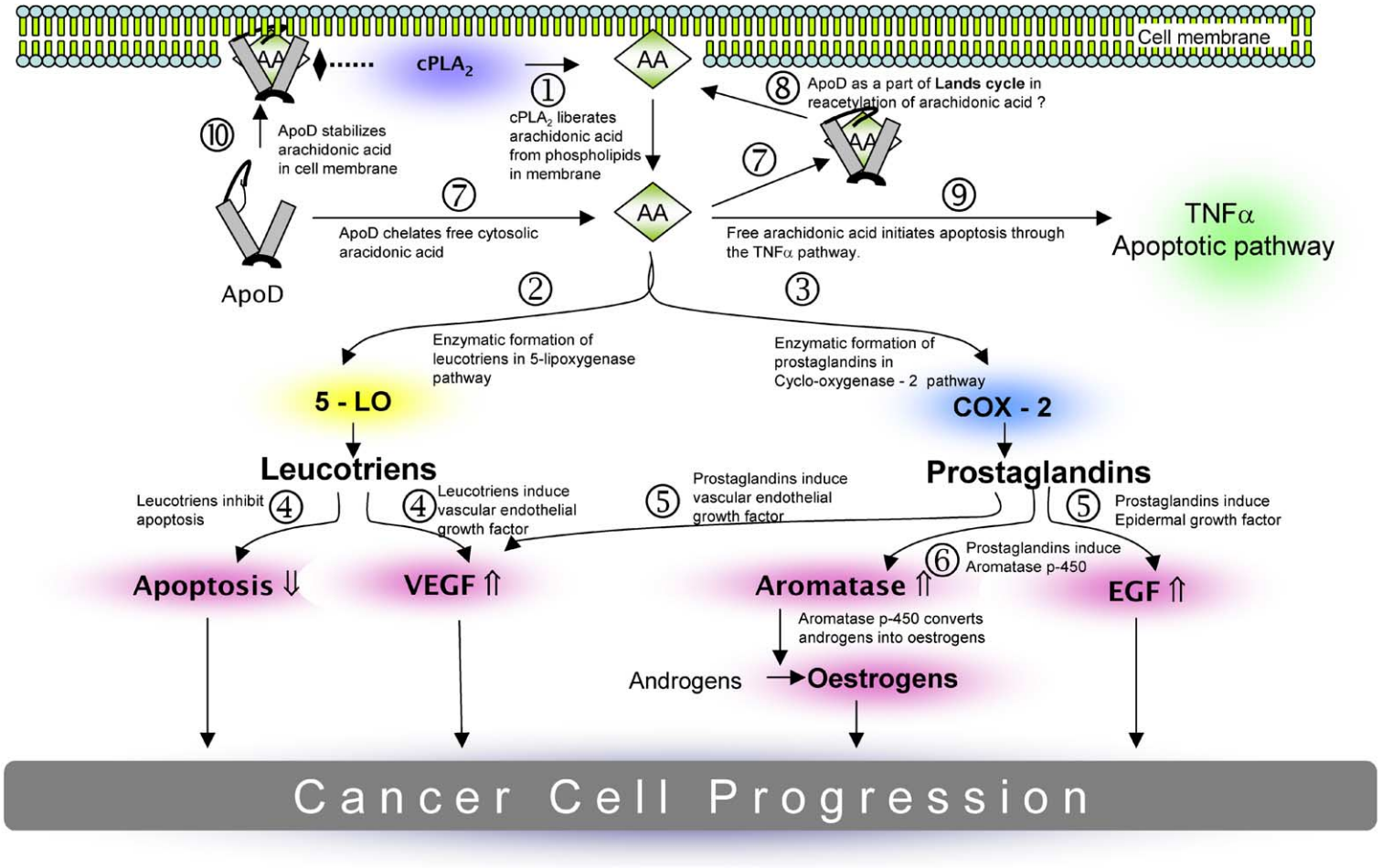


Fig. 5. Importance of ApoD in arachidonic acid (AA) signaling pathways. Adapted from references [73,99] and [100]. The main message in the figure is that ApoD could be a factor that has a strong influence on the availability of free arachidonic acid in the cell. The numbers indicate the sequence of different reactions/interactions. Abbreviations: 5-LO: 5-lipoxygenase; AA: arachidonic acid; $cPLA_2$: cytoplasmic phospholipase A_2; COX-2: cyclo-oxygenase 2; ER: oestrogen receptor; EGF: epidermal growth factor; TNFα: tumour necrosis factor alfa; VEGF: vascular endothelial growth factor. 1. $cPLA_2$ cleaves the binding between glycerol and AA and liberates AA into the cytoplasm. 2. AA is the 5-LO enzyme substrate. 3. AA is the COX-2 enzyme substrate. 4. Leucotriens increase VEGF pathway in neoangiogenesis and inhibit apoptosis. 5. Prostaglandins crosstalk with the EGF pathway and neoangiogenesis via the VEGF pathway. 6. Prostaglandins stimulate transcription of aromatase and crosstalk with the ER pathway. 7. Apo D binds/chelates free AA in the cytosol and removes it from the free functional substrate pool of the enzymatic pathways described in 2 and 3. 8. ApoD may be part of the Lands' cycle, where free AA is esterified back in the phospholipids bilayer membrane in order to keep a low level of free arachidonic acid in the cytoplasm. 9. Increased free arachidonic acid is toxic for the cell and initiates apoptosis through the TNF-α pathway. 10. ApoD protects liberating of AA in the plasma membrane against the enzymatic cleavage function of $cPLA_2$.

ways. COX-2 activity in cancer correlates with enhanced invasiveness and cell motility [88]. It is therefore understandable that in ER-negative breast cancer, COX-2 correlates with poorer disease-free and disease-related survival [105]. Cross-talk between the COX-2 pathway and other pathways is thought to be important, including involvement of vascular endothelial growth factor receptor [80], the epidermal growth factor receptor pathways [30], and the aromatase/ER pathways [10]. Hypothetically this gives ApoD an important role in the control of cancer cell progression. Intriguingly, free arachidonic acid is pro-apoptotic, and ApoD will tend to facilitate its re-acetylation in tumour cells that have become dependent on prostaglandin signalling pathways. ApoD thus could reduce the apoptotic "drive" from arachidonic acid. In cell senescence, ApoD might contribute to avoid apoptosis by chelating all free arachidonic acid.

6. ApoD and relationship with sex steroid hormone receptors

The sex steroid receptors oestrogen receptor (ER), progesterone receptor (PR) and androgen receptor (AR) belong to the nuclear receptor superfamily and each exists in two isoforms, ERα/ERβ, PRA/PRB and ARA/ARB respectively. Their effects are mediated through both a slow nuclear/genomic [67] and a fast non-genomic/membrane-initiated steroid signalling (MISS) pathway [56].

6.1. Androgens, and androgen receptor (AR)

Androgens act through both membrane-bound [36] and intracellular AR localized in the cytoplasm and nucleus [22]. ApoD transcription is highly up-regulated in cancer cell lines when androgens are introduced [85,87,96]. However, in contrast to the cell culture studies, no association was found between AR and ApoD in breast cancer when analyzed by immunohistohemistry on serial sections [34]. The strong up-regulation of ApoD by androgens must therefore be modified by other factors, meaning that the micro-environmental difference between cell cultures and solid tumours (the absence or presence of stromal cells) becomes of interest for the study of ApoD and AR interaction. Stromal cells produce aromatase which converts androgens into oestrogens [98]. These have a strong inhibiting effect on ApoD transcription. This finding could explain the discrepancy between epithelial cancer cell cultures and solid tumours regarding AR and ApoD. In evaluating data from the literature about the interaction of ApoD with steroid and steroid receptors, it is essential to consider the study model used.

Thus, ApoD is upregulated by androgens and down-regulated by oestrogens. ApoD can be a marker of the balance between AR, aromatase and ER.

6.2. Progesterone receptor (PR) and oestrogen receptor (ER) pathways

ApoD has a strong affinity for progesterone [48], which indicates an important role for ApoD in the PR pathway very likely (Fig. 6). The destiny of progesterone within an ApoD positive cell is uncertain, but may have two effects on the PR pathways. The first effect of ApoD might be to reduce the biological availability of progesterone to the genomic PR pathway by binding progesterone and diverting it to metabolic inactivation. Because the progesterone–PR complex in the nucleus has a short half-time [95] and ApoD is mainly located in the cytoplasm [110], the overall result may be reduction of the effect of progesterone. The second effect on the progesterone pathway is that ApoD may serve as a cytoplasmic reservoir for progesterone, creating a "slow release" and prolonged genomic progesterone effect. Moreover, a cytoplasmic reservoir might switch progesterone from the genomic pathway to the non-genomic MISS pathway (Fig. 6). The effect of either of these proposed pathways would depend on the relative content of the two isoforms, PRA and PRB [14], which have opposite functions. While PRA inhibits transcription, PRB activates transcriptional function on progesterone response elements in target genes [103] (Fig. 6). Because of these opposite effects, the net effect of progesterone depends on the PRA : PRB ratio in the cell [60]. Thus, if progesterone enters an ApoD-negative cell, it can reach the nucleus, bind to PR, and exert its effects without any interaction. In contrast, in cytoplasmic ApoD positive cells, progesterone could be chelated, stored or metabolized.

In the genomic pathway, binding of oestradiol to ER-α increases proliferation, while binding to ER-β decreases proliferation [67]. In breast cancer, nuclear ER-α is often over-expressed and functions as an oncogene [107]. Hence, competitive blocking of the slow nuclear ER pathway with tamoxifen is a well-known targeted therapy in breast cancer and so, the ER-α MISS pathway [51,56] could explain tamox-

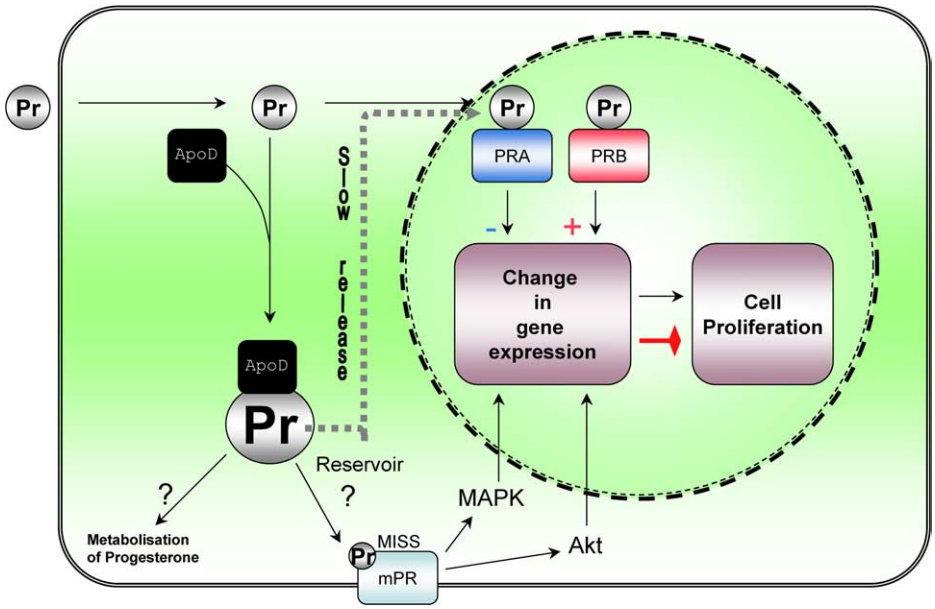


Fig. 6. Two models showing how ApoD may interact with progesterone. 1. In the cytoplasm ApoD may bind progesterone and divert it to metabolic degradation. This will reduce the effect of progesterone in both the genomic and MISS pathway. 2. ApoD binds free progesterone and hides it away from metabolisation. This will create an intracellular reservoir of progesterone and a "slow release" effect (⇢) on the genomic pathway may occur. Also, this may enhance (→) the MISS progesterone pathway. See text for further discussion. Abbreviations: ApoD: Apolipoprotein D; MAPK: mitogen activated protein kinase; mPR: membrane progesterone receptor; Pr: progesterone; PRA: progesterone receptor A; PRB: progesterone receptor B.

ifen resistance by downstream phosphorylation of target proteins in the MAPK and Akt pathways and increase proliferation in breast cancer [68,91]. Down-regulation of ApoD via the genomic ER-α pathway is potent and sensitive [19] (Fig. 3). Combined with the ability of ApoD to inhibit MAPK translocation into the nucleus, the genomic inhibition of ApoD may enhance the effect through the ER-α MISS pathway (Fig. 4). Furthermore, since ApoD has three oestrogen response elements in its promoter region exerting a strong inhibitory effect on gene transcription, co-expression with ER could be a sensitive marker for a non-functional genomic ER-α pathway.

7. ApoD: A marker of cellular senescence and homeostasis

Recent studies indicate that ApoD is involved in both the cell senescence program and homeostasis [9,24,106]. Increased ApoD expression is associated with a marked reduction in cell proliferation [7,53,55, 85–87,96]. Cellular senescence induced by starvation in human oral keratinocytes [40] and fibroblasts [74] leads to a striking increase in ApoD expression and indicates participation in the cell senescence program [5]. Thus, ApoD may be a marker for cell senescence. Generally, a senescent G_0 tumour cell apparently benefits cancer patients [12]. Interestingly, however, cell senescence of the adjacent tumour stroma is prognostically disadvantageous [13]. The following molecular cell biological facts may explain this apparent discrepancy. Non-senescent stroma secretes tissue inhibitors of metalloproteinases and serpins, which hampers cancer cell invasion [1]. On the other hand, senescent stroma secretes high levels of specific matrix metalloproteinases (MMPs) [40], epithelial growth factor [40], and inflammatory cytokines, which promotes cancer cell invasion and metastases [13,70]. When ApoD overexpression occurs in the stromal cells, senescence favours the epithelial invasive cancer cells, thereby worsening the prognosis of cancer patients. Also, the proteolytic properties of ApoD itself may add to the collagen dissolvement by the matrix metalloproteinases. Alternatively, ApoD might form complexes with matrix metalloproteinases and enhance their effects. It is therefore clear that the functional significance of ApoD expression in epithelial cancer cells or in the adjacent stroma has two widely different effects. This dichotomy supports the expression, *"ApoD*

is a good citizen but a bad neighbour" (rephrased from [13]). This difference also holds in the pancreas, where juxta-tumoural expression of ApoD is an important response marker for tumour invasion into the adjacent stroma [38] regardless of tumour type [76]. Moreover, in prostatic bone metastasis, ApoD is upregulated at the tumour–bone interface together with MMP-7 [57].

Interestingly, Leung et al. [50] found an increase in both human and rat aortic smooth muscle motility when ApoD transcription was induced. Conversely, the motility was reduced when ApoD transcription was inhibited which was linked to increased Rac-1 activation. ApoD can be expressed in or taken up from the micro-environmental neighbouring cells into smooth muscle cells and can modulate their motility in response to growth factors [50]. If the same happens in cancer cells, this could imply that ApoD expression in the surrounding stroma is a poor prognostic sign because it results in the sustaining of cancer cell motility and invasion.

ApoD interacts with leptins. This means that a network of crosstalking, signalling pathways are activated and leptins are considered to play an important role in the relationship between nutrition, obesity, cardiovascular disease and cancer [17,26,37,39, 46,79,102]. This has been described in detail elsewhere [37,52] and can indicate that ApoD is involved in the micro-environmental effects of fat tissue adjacent to the breast cancer cells.

8. Observations in breast cancer

In the breast, ApoD expression may play a role in local steroid production and intracellular storage of the ligand molecules (i.e. progesterone and arachidonic acid). In fluids from fibrocystic breast disease, ApoD is found in up to 800–1000 times the amount found in plasma [49], and over 50% of invasive breast cancers are ApoD positive [18,49,93]. ApoD has often been measured biochemically in the tumour cytosol [84,93], which is a mixture of stromal and tumour cells where tumour/stroma differentiation cannot be made. Studies that have localized and quantified ApoD separately in the epithelial and stromal parts of the tumour found that ApoD was prognostic [18,43,104], whereas another study using the cytosol method did not [93]. This distinction indicates that ApoD determination in cytosol is a less reliable prognosticator than immunohistochemically determined ApoD [18].

By cytosol determined ApoD, a higher ApoD content is observed in adjacent normal tissue than in the primary tumour in the same breast [94]. ApoD content is highest in benign tumours [49] whereas it is lower in invasive cancer and further decreases with higher stages and metastatic cancer. Moreover, the proportion of patients with ApoD-negative tumours was higher in patients with at least four positive axillary lymph nodes when compared to those with fewer than four involved nodes [43,49,92,93]. Well-differentiated breast cancers show the highest levels of ApoD and lower levels are found high grade cancers [18,82–84]. ApoD is expressed more frequently in invasive lobular than in invasive ductal, carcinomas [82] and more often in postmenopausal and elderly than in premenopausal or younger patients [18]. The explanation for this is probably the postmenopausal decline in circulating oestradiol. As a result, the suppression of ApoD transcription by oestradiol is decreased and hence ApoD protein increased.

ApoD expression in primary epithelial breast cancer cells has been claimed to be associated with a *favourable prognosis*. When ApoD is overexpressed in the *pre-existent stromal cells*, senescence favours the epithelial invasive cancer cells by secreting high levels of MMPs, epidermal growth factor and inflammatory cytokines. ApoD expression in the adjacent tumour stroma is, thus, a *poor prognostic marker* in breast cancer [104]. The location of ApoD within, versus around the tumour therefore should be taken into consideration when evaluating results from studies on ApoD.

Progesterone receptor as usually determined in breast cancer is a mixture of PRA and PRB, which have opposite effects. Consequently, PR determination without distinguishing PRA and PRB is not very specific and as a result, the prognostic significance of interactions between ApoD, PR and progesterone in breast cancer has not yet been studied in a satisfactory manner. In normal breast tissue, PRA and PRB are equally expressed [28,62]. However, in breast cancer, the normal co-expression of PRA and PRB is lost, and the ratio is increasingly skewed [29,62] toward PRA as the cancers become more malignant [60] (less cohesion, decreased contact inhibition, increased cell motility, and metastatic potential). As a result, an increased PRA : PRB ratio is associated with a poor prognosis. This shift in signalling is prominent in steroid-independent breast cancer cells and is also observed in tumours that are dependent on growth factor signalling [45,77].

The relationship between ER, tamoxifen and ApoD is perhaps one of the most promising aspects of ApoD

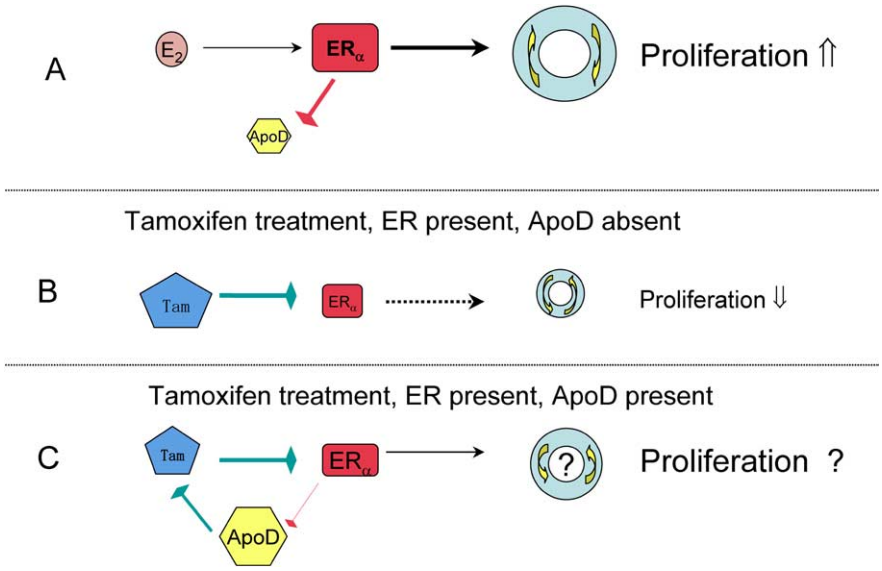


Fig. 7. The triangular relationship between tamoxifen, ER and ApoD. (A) ER has a stimulatuory effect (⟶) on the cell proliferation, and an inhibitory effect (⟶) on ApoD transcription. (B) Administration of tamoxifen will inhibit (⟶) ER action and reduce proliferation. (C) In ApoD positive cells, the ER is either not functional and will not exert its potent inhibitory effect on ApoD transcription or tamoxifen will increase ApoD transcription by reducing the inhibitory ER effect (⟶). ApoD may in turn bind and chelate tamoxifen (⟶). In either case, tamoxifen will probably not have the same antiproliferative effect compared with a ApoD negative cell. Abbreviations: ApoD: Apolipoprotein D; Tam: tamoxifen; E_2: oestradiol; ER: oestrogen receptor.

in breast cancer. Although one early report [84] indicated a positive correlation between ApoD and ER activity, an inverse relationship between the two has been reported in later studies [41,49,92] as expected from cellular studies discussed above. Moreover, ApoD is a predictor of adjuvant tamoxifen treatment in node-positive breast cancer patients with oestrogen receptor (ER)-positive tumours. Interestingly, only patients with ApoD-negative tumours had improved relapse-free survival when adjuvant tamoxifen treatment was given for 2 years; in the same group of patients with ApoD-positive tumours, the outcome was not influenced by adjuvant tamoxifen treatment [93].

The triangular relationship between ER, tamoxifen and ApoD opens up an interesting explanation for this observation. Firstly, since ApoD expression may indicate a non-functional ER-pathway, tamoxifen will not be effective in the subset of ER-positive/ApoD-positive cancers [93]. Secondly, ER suppresses ApoD transcription, and when tamoxifen is administered, this suppression is lost and ApoD transcription increases (Fig. 7). The binding affinity ApoD has to tamoxifen (40% of progesterone) may then sequester tamoxifen and mitigate its biological effects. Hence, a tamoxifen/ER/ApoD equilibrium could exist.

Conclusively, according to the above considerations, ERα positive /ApoD positive tumours should not receive tamoxifen, but rather aromatase inhibitors. This will reduce the local oestradiol production, reduce ER stimulation (both nuclear and MISS pathway) and thereby reduce proliferation. Of course, this hypothesis must be verified in appropriate studies.

9. Conclusions

Apolipoprotein D interacts with many important pathways, e.g., MAPK, leptin, Arachidonic Acid and Progesterone. ApoD has several likely mechanistic functions (Fig. 8):

1. Building up an intracellular reservoir of an unstable ligand;
2. To chelate (bind and divert) the ligand either into an alternative pathway or to its metabolism locations;
3. Making interactions with transmembrane receptors;
4. Participate in recycling of membrane bound molecules/ligands;
5. Endosomal trafficking;
6. Making complexes with other molecules either extracellularly or intracellularly.

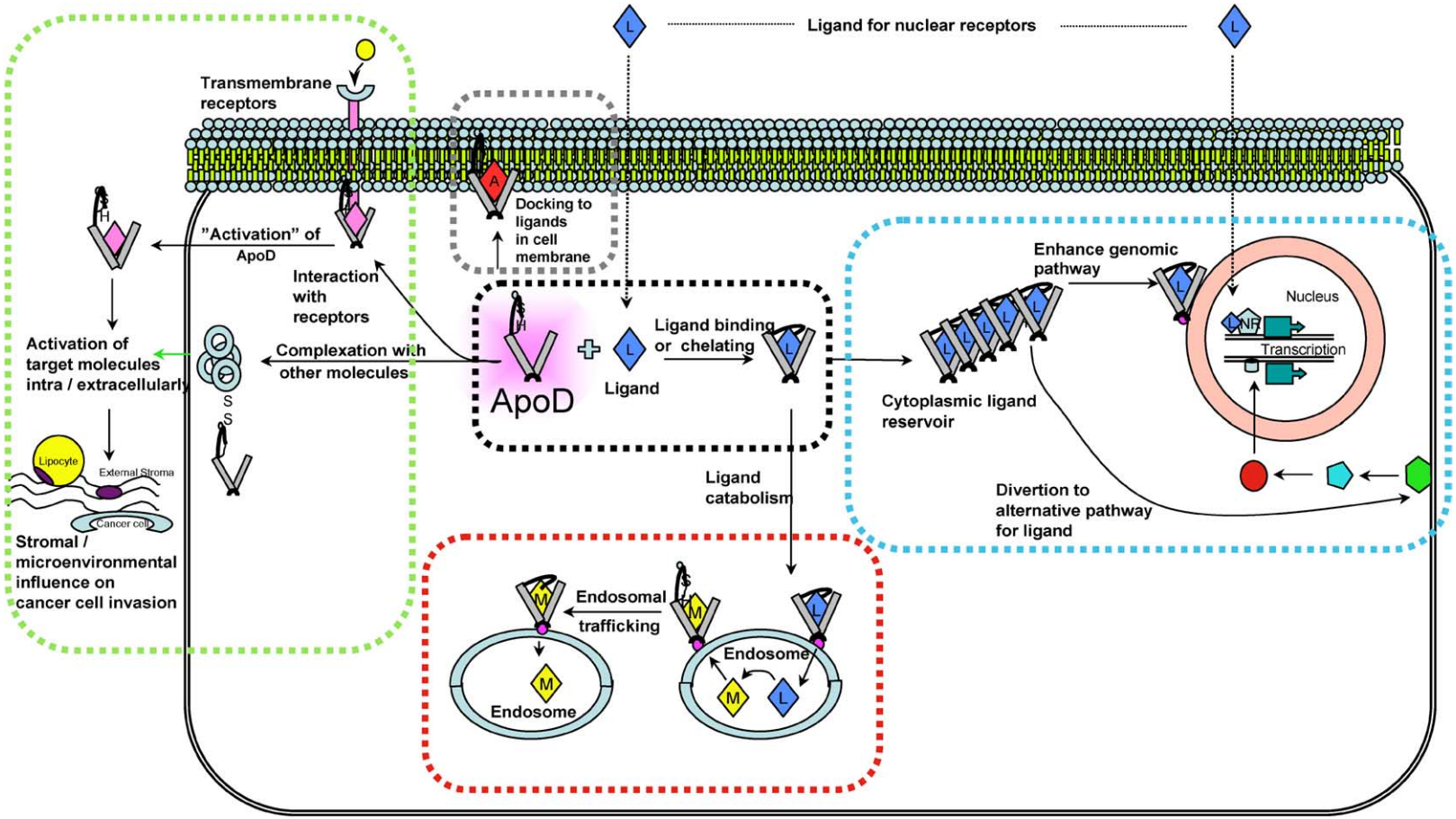


Fig. 8. General mechanistic model of cellular actions of ApoD. ApoD may be produced locally in a cell, secreted to or taken up from juxta cellular micro-environment. Black dotted rectangle: In the cell, generally ApoD may bind hydrophobic ligands. Blue dotted rectangle: The ligand bound to ApoD may be built up as an intracellular reservoir that can increase the effect on gene transcription of nuclear receptor ligands. Alternatively this cytoplasmic reservoir may divert the ligand to alternative pathways. Red dotted rectangle: ApoD is able to exert endosomal trafficking, and may take part in chelating and diverting ligands to its metabolic pathways. This may also include drugs administered. Grey dotted rectangle: ApoD is also able to dock to cellular membrane molecules and may stabilize these molecules and hence be a regulator of the turnover of such molecules. Green dotted rectangle: Moreover, possible interactions with transmembrane receptors are suggested to take place and ApoD may form complexes with other molecules either inside or outside the cell that are involved in the micro-environment of the cancer cell. Abbreviations: L in blue diamond: ligand for nuclear receptors; M in yellow diamond: metabolite of ligand (L); A in red diamond: intramembranous ligand.

These functions may be tissue specific, but within a certain tissue, ApoD may also have different roles in both the normal/physiological state and pathological state [109]. Thus, it seems evident that ApoD plays a central role in humans both in normal- and in pathophysiological pathways, including disturbances in fat metabolism and cancer progression. Up-regulation of ApoD in tumour cells and adjacent stroma has opposite effects. Consequently, *in situ* determination of ApoD by immunohistochemistry should be the preferred method of analysis. Previously employed methods based on cytosol quantification are most likely inaccurate and provide less biological information of interest. In breast cancer, ApoD may evolve as an interesting prognostic and predictive factor in decision making for tailored adjuvant (endocrine) therapy. Special attention should be paid to the triangular relationship of tamoxifen/ER/ApoD.

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Abbreviation	Explanation
⊣	inhibitory effect
→	stimulatory effect
5-LOX	5-lipoxygenase
AA	arachidonic acid
ApoD	apolipoprotein D
ARA	Androgen receptor A
ARB	Androgen receptor B
COX 2	cyclo-oxygenase 2
$cPLA_2$	cytoplasmic phospholipase A_2
E_2	oestradiol
EGF	epidermal growth factor
ER	oestrogen receptor
ER-α	oestrogen receptor α
ER-β	oestrogen receptor β
GCDFP-24	gross cystic disease fluid protein-24
MAPK	mitogen-activated protein kinase
MISS	membrane-initiated steroid signalling
MMP	Matrix metalloproteinase
mPR	membrane bound progesterone receptor
NR	nuclear receptor
PBC	progesterone binding component
PBCP	progesterone binding cyst protein
PBP	progesterone binding protein
Pr	progesterone
PR	progesterone receptor
PRA	progesterone receptor A
PRB	progesterone receptor D
RTK	receptor tyrosine kinase
TAM	tamoxifen
TNF-α	tumor necrosis factor α
VEGF	vascular endothelial growth factor

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