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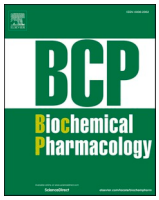

Review

AEBS inhibition in macrophages: Augmenting reality for SERMs repurposing against infections

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ABSTRACT

Beyond their clinical use as selective estrogen receptor modulators (SERMs), raloxifene and tamoxifen have attracted recent attention for their favorable activity against a broad range of dangerous human pathogens. While consistently demonstrated to occur independently on classic estrogen receptors, the mechanisms underlying SERMs antimicrobial efficacy remain still poorly elucidated, but fundamental to benefit from repurposing strategies of these drugs. Macrophages are innate immune cells that protect from infections by rapidly reprogramming their metabolic state, particularly cholesterol disposal, which is at the center of an appropriate macrophage immune response as well as of the anabolic requirements of both the pathogen and the host cells. The microsomal antiestrogen binding site (AEBS) comprises enzymes involved in the last stages of cholesterol biosynthesis and is a high affinity off-target site for SERMs. We review here recent findings from our laboratory and other research groups in support of the hypothesis that AEBS multiprotein complex represents the candidate pre-genomic target of SERMs immunomodulatory activity. The cholesterol restriction resulting from SERMs-mediated AEBS inhibition may be responsible for boosting inflammatory and antimicrobial pathways that include inflammasome activation, modulation of Toll-like receptors (TLRs) responses, induction of interferon regulatory factor (IRF3) and nuclear factor erythroid 2-related factor 2 (NRF2)-mediated transcriptional programs and, noteworthy, the mitigation of excessive inflammatory and proliferative responses, leading to the overall potentiation of the macrophage response to infections.

1. Introduction

It is well established that the microsomal antiestrogen binding site (AEBS) is an estrogen receptor (ER)-unrelated high affinity site for selective estrogen receptor modulators (SERMs) with demonstrated involvement in cell growth control [1–3] and that it is formed by the 3β-hydroxysterol-Δ8-Δ7-isomerase or D8D7I (also named emopamil-binding protein or EBP), the 3β-hydroxysterol-Δ7-reductase or DHCR7, and the 3β-hydroxysterol-Δ24-reductase or DHCR24 [4,5] (Fig. 1A). These enzymes are interconnected in a metabolic chain that

Abbreviations: AEBS, antiestrogen binding site; Akt, AKT serine/threonine kinase; AY9944, *trans*-1-4-bis{chlorobenzylaminomethyl}-cyclohexane)dihydrochloride; Btk, Bruton's tyrosine kinase; ChEH, cholesterol-epoxide hydrolase; COVID-19, coronavirus disease 2019; CT, cholestane-3β,5α,6β-triol; D8D7I, 3β-hydroxysterol-Δ^8-Δ^7-isomerase; 7-DHC, 7-dehydrocholesterol; DHCR7, 3β-hydroxysterol-Δ^7-reductase or 7-dehydrocholesterol reductase; DHCR24, 3β-hydroxysterol-Δ^{24}-reductase or 24-dehydrocholesterol reductase; 7-DHD, 7-dehydrodesmosterol; EGFR, epidermal growth factor receptor; 5,6-EC, 5,6-epoxycholesterol; ER, estrogen receptor; HIV-1, human immunodeficiency virus; IFN, interferon; IL, Interleukin; IRF, interferon regulatory factor; LPS, lipopolysaccharide; NLRP3, NLR family pyrin domain containing 3; NRF2, nuclear factor erythroid 2-related factor 2; PI3K, phosphoinositide 3-kinase; P2X7R, P2X7 receptor; raloxifene, [6-hydroxy-2-(4-hydroxyphenyl)-1-benzothiophen-3-yl]-[4-(2-piperidin-1-ylethoxy)phenyl]methanone; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SCAP, SREBP cleavage-activating protein; SERMs, selective estrogen receptor modulators; SREBP2, sterol regulatory element-binding protein 2; STING, stimulator of interferon genes; tamoxifen, 2-[4-[(Z)-1,2-diphenylbut-1-enyl]phenoxy]-N,N-dimethylethanamine; TBK1, TANK-binding kinase 1; TLR, Toll-like receptor; TNFα, Tumor Necrosis Factor α; U18666A, 3-β-[2-(diethylamino)ethoxy]androst-5-en-17-one.

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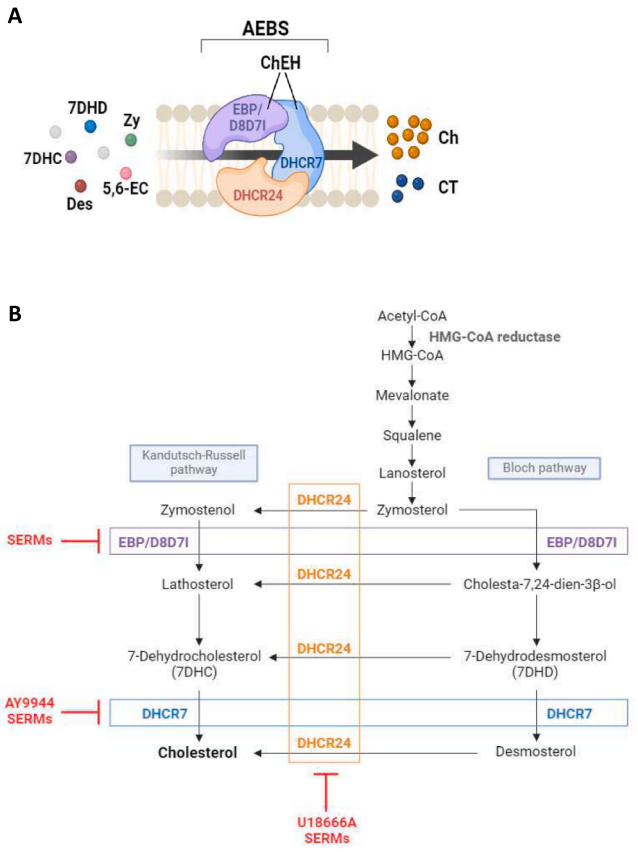

Fig. 1. Implication of AEBS in cholesterogenesis. Panel A) AEBS complex is represented by the association of EBP/D8D7I, DHCR7 and DHCR24, with EBP/D8D7I and DHCR7 that are subunits of the epoxide hydrolase specific for 5,6-epoxycholesterol (5,6-ECs) or cholesterol epoxide hydrolase (ChEH). ChEH catalyzes the hydration of 5,6-EC oxysterols (α and β diastereoisomeric products of cholesterol auto-oxidation by reactive oxygen species on the delta 5 double bond) to produce cholestane-3β,5α,6β-triols (CTs). **Panel B)** Schematic diagram of the multienzymatic process of cholesterogenesis that begins with the mevalonate pathway leading to lanosterol, which can enter into either the Bloch or the Kandutsch-Russell pathway to produce cholesterol via desmosterol or 7-dehydrocholesterol (7-DHC), respectively. EBP/D8D7I shift the C8-9 double bond to the C7-8 position in the B-ring of sterols and DHCR7 removes the C7-8 double bond in 7-dehydrodesmosterol (7-DHD) and 7-DHC in the penultimate and ultimate steps of the two post-lanosterol arms to form cholesterol. DHCR24 is essential for the Kandutsch-Russell arm as it reduces the C24-25 double bond on the side chain of sterol intermediates and synthesizes cholesterol from desmosterol in the last step of the Bloch pathway. Various inhibitors that block specific post-lanosterol enzymatic steps involving EBP/D8D7I, DHCR7 and DHCR24 are depicted in red. Created with BioRender.com.

ensures the efficient and concerted control of late stages of cholesterol biosynthesis [6], whereas D8D7I and DHCR7 form the microsomal cholesterol-epoxide hydrolase (ChEH) heterodimeric complex [4,7] supporting the notion that AEBS bears different functionalities. The genetic and protein classifications are summarized in Table 1.

AEBS is concentrated in the endoplasmic reticulum membranes of different cells [8–10]. Accordingly, subcellular fractionation, immunocytochemistry, and overexpression studies locate D8D7I, DHCR7 and DHCR24 within the network of endomembranes, mainly in the endoplasmic reticulum membranes, but also in the contiguous nuclear envelope and in the Golgi apparatus [11–14] and are almost ubiquitous in normal cells of mammals, reflecting the critical role played by cholesterol in cellular pathways [15].

Literature data suggest that the primary molecular target of SERMs is the isomerase [16] and, indeed, tamoxifen has been reported to physically interact and inhibit D8D7I [17,18] (Fig. 1B). Furthermore, sterol

Table 1
The hetero-oligomeric AEBS complex is composed of at least three enzymes that give rise to different functionalities. Names and identifiers of proteins and genes, together with chromosomal localization are detailed (Homo sapiens).

Gene	*Gene location*	Protein	EC number
EBP	*Xp11.23*	Emopamil Binding Protein or 3β-hydroxysterol-Δ8-Δ7-isomerase (EBP/D8D7I)	5.3.3.5
DHCR7	*11q13.4*	7-Dehydrocholesterol Reductase or 3β-hydroxysterol-Δ7-reductase (DHCR7)	1.3.1.21
–	–	Cholesterol-Epoxide Hydrolase (ChEH)	3.3.2.11
DHCR24	*1p32.3*	24-Dehydrocholesterol Reductase or 3β-hydroxysterol-Δ24-reductase (DHCR24)	1.3.1.72

Source: Gene Cards, OMIM, UniProtKB

intermediate profile analyses indicate that SERMs may also target either DHCR7 or DHCR24 [5,16,19], opening up the possibility that AEBS activity may be variably influenced by the different chemical structures of SERMs. Among other inhibitors of AEBS are a variety of chemically different drugs including opioid analgesics, antidepressants, antipsychotics, fungicides, and immunosuppressants [20,21]. Interestingly, identification of structural elements necessary for AEBS binding led to the synthesis of selective and high affinity inhibitors, the diphenylmethane derivatives of tamoxifen, which are devoid of ER binding activity [2,22,23]. Collectively, AEBS is to be considered a multidrug binding-protein assembly representing a target for several drugs exerting their activities through the perturbation of cholesterol homeostasis at its biogenesis level.

Exploitation of SERMs off-target mechanisms represented an opportunity for repurposing these medications in proliferative-related clinical conditions. Beyond ER-negative cancer [24] and fibrotic disease [25], SERMs are indeed showing promising *in vitro* and *in vivo* effects against a broad range of human pathogens [26,27]. In this novel scenario, an intriguing viewpoint has emerged that conceives SERMs as direct regulators of macrophages, that quickly modify intracellular metabolic pathways, particularly cholesterol availability, to mount an appropriate anti-infective response [28–30]. Analyses from our laboratory predict the expression of D8D7I, DHCR7 and DHCR24 in different primary macrophages of mouse and human origin (unpublished results).

The purpose of this review is therefore to discuss evidence from the literature and recent data from our laboratory in favor of the hypothesis that AEBS may be the pre-genomic target that SERMs activate in macrophages to boost the host immune defense.

2. SERMs and infections

SERMs are neatly appearing as therapeutic options against infectious diseases and the burden of antimicrobial resistance, in monotherapy or in association with conventional antimicrobial chemotherapeutics [27,31–35]. Recent computational or experimental screening assays provide strong support to the use of SERMs as anti-infective candidates, particularly against (re)emerging infectious diseases [36], including Zika [37], Mycobacterium [38], as well as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [39,40]. Interestingly, raloxifene has attracted substantial attention for treatment of SARS-CoV-2 infection due to its high pulmonary distribution and antiviral activity [41,42] associated with a shortened time of SARS-CoV-2 shedding in coronavirus disease 2019 (COVID-19) patients [43].

The mechanism of SERMs antimicrobial activity has only begun to be explored and points to the involvement of macrophages as primary determinants of the outcome of infections.

2.1. SERMs activity in macrophages

Macrophages are ubiquitous innate immune cells endowed with the

ability to quickly adapt their metabolism to mount an appropriate immune response and destroy invading pathogens [44]. Under microenvironmental signals, macrophages acquire different functions characterized by distinct expression profiles of immune proteins and metabolic enzymes with two extreme immune phenotypes, namely the inflammatory or M1 phenotype and the anti-inflammatory or M2 phenotype [45]. Upon infection, M1 activation leads to the rapid and massive production of inflammatory and microbicidal mediators, phagocytosis of invading pathogens and antigen presentation to lymphocytes. On the other hand, M2-activated macrophages exert immunosuppressive activities, downregulate inflammation and promote tissue repair. Thus, macrophages are able to dynamically reprogram their gene expression profile in order to attack foreign invaders and amplify immune competence, as well as to dampen immune reactivity and restore the physiological conditions. As a consequence, the balance guarantees homeostasis and imbalance produces chronic inflammation, tissue damage and disease [46].

Macrophage phenotypic activation can be affected by both estrogens and their pharmacological modulators. ERα agonists are assumed to accelerate M1 activation to fasten tissue repair [47–49], whereas low concentrations of SERMs were reported to antagonize estrogenic immune effects. Importantly, higher drug levels, such as those used in clinical off-target indications, triggered ER-independent cytoprotective effects, phagocytosis and a specific M1 response to the bacterial signal, lipopolysaccharide (LPS) [50–54].

Also considering that SERMs were shown to be devoid of intrinsic bactericidal [31,55] or virucidal activities [37,51], available observations lend ground to support a novel hypothesis, for which the anti-infective activity of SERMs mainly occurs through the interaction with macrophage off-target receptors that potentiates their immune ability [27,51,56–59].

2.2. *Non-conventional mechanisms of SERMs immune activity in macrophages*

Although being chemically distinct entities, SERMs share a cationic amphiphilic nature that allows them to easily enter *endo*-lysosomes, where they become protonated by the acidic environment and acquire a polar structure that hinders their diffusion to the cytoplasm [60]. The *endo*-lysosomal accumulation of these molecules results in pH alteration and destabilization of the structure, composition and function of lysosomal membranes in different cell types including macrophages [61–63], with a relevant impact on the life cycle of intracellular pathogens that also rely on cellular *endo*-lysosomes for spreading inside the host cells [51,64–66]. Cholesterol-rich membrane microdomains, known as lipid rafts, have also been implicated in the fusion of microbial proteins and membranes with the host counterpart, favoring the intracellular spread of the microbial genome [67–69]. The perturbation of cholesterol levels within lipid rafts has been proposed to mediate the suppression of enveloped virus infections induced by SERMs and other intracellular cholesterol-reducing agents in different cell types, through the inhibition of the virus-cell fusion and viral genome intracellular egression [65,70–73]. Also the protective effects of SERMs against prion disease have been ascribed to the modification in cholesterol composition at lipid rafts, resulting in the activation of lysosomal enzymes that degrade prion proteins [74,75]. Considering the multiplicity of the affected pathogen species, it seems unlikely that SERMs find their specific targets in viral fusion peptides or their host cell receptor counterparts, as these are widely dissimilar among microbial species. Instead, a change in cholesterol composition within host lipid rafts seems a more conceivable mechanism to create an unfavorable microenvironment for pathogen intracellular spread and replication. Whether AEBS is physically or functionally associated with lipid rafts still needs to be clearly defined. Nevertheless, membrane instability and lysosomal damage would not be so productive in terms of anti-microbial activity if SERMs did not induce a parallel, highly specific pathway that, from the reduction in cholesterol biogenesis induced by AEBS inhibition, leads to inflammation and immune reprogramming, also considering that bearing a cationic amphiphilic structure is not sufficient for a compound to produce anti-infective effects [76].

Another important aspect of SERMs activity is their effects on cell proliferation and death. In fact, the pioneering work by Tang et al. originally implicated AEBS in the antiproliferative effect of SERMs in non-estrogen target cells; interestingly, tamoxifen action was dose-dependently reversed by cholesterol, but not by mevalonate, pointing to a target in the late stage of cholesterol biosynthesis [77]. Consistent with the metabolic demand of proliferating cells, AEBS was indeed found to be expressed in ER-positive and −negative cancer cell lines and human cancer specimens [78–81] and micromolar SERMs concentrations associated with cell death in cancer and other cell types [2,82,83]. Remarkably SERMs were reported to induce anti-proliferative effects in macrophages without inducing cell death; rather, they improve cell competence in boosting immune responses while maintaining cell viability [53,54].

3. Anti-infective potential of macrophage AEBS inhibition

An alternative and more conceivable as well as exploitable pharmacological target of SERMs anti-infective activity, among others [27], is represented by the AEBS multiprotein complex, in that: i) it is a high affinity binding site for SERMs, probably the only ER-unrelated target endowed with this property; ii) its inhibition alters macrophage metabolism and stimulates highly pervasive and long-lasting immune responses; iii) its blockage also concurs in limiting the propagation of intracellular pathogens.

Inhibition of AEBS by SERMs is expected to lead to the rapid accumulation of sterol precursors and the reduction in cholesterol and cholestane-3β,5α,6β-triols (CTs) in cells (Fig. 1A), as observed in SERMs-treated liver and cancer cell lines [2,84–86]. In the upcoming sections we will examine the concept that this known SERMs-mediated modification of sterol metabolism via AEBS contributes not only to their antiproliferative pharmacology but also to the boosting of macrophage-driven host defense against infections; as well, we will discuss the identity and function of downstream mediators that accomplish macrophage activation.

3.1. *AEBS as therapeutic target against infections*

Since the initial reports on ER-independent activity of SERMs against the human immunodeficiency virus (HIV-1) and Moloney murine leukemia virus, numerous evidence emerged that implicated the inhibition of AEBS components as a beneficial pharmacological attempt in the host-pathogen contention [87,88]. Experimental approaches that encompass the use of pharmacological inhibitors, the administration of cholesterol precursors, like 7-dehydrocholesterol (7-DHC), or the genetic manipulation of AEBS enzymatic subunits, have later substantiated the correlation between AEBS inhibition and antimicrobial activity in cell and animal models. As summarized in Table 2, most of the data points to a relevant anti-infective efficacy of DHCR7 inhibition against pathogen infections. Indeed, DHCR7 inhibition obtained by treatments with the pharmacological inhibitor AY9944 (Fig. 1B) or the 7-DHC precursor or by DHCR7 gene silencing *in vitro* as well as in infected mice, reduced enterovirus infectivity, while DHCR7 gene overexpression stimulated viral replication [89]. Alongside, DHCR7 indirect inhibition triggers innate immune responses to highly pathogenic coronavirus [90] and its pharmacological inhibition has immunostimulatory effects and promotes resistance to HIV-1 and opportunistic infections [91] as well as several other viral species [92–94].

No less important are the data on DHCR24 blockade obtained by pharmacological inhibition with U1666A (Fig. 1B) or gene silencing/knockout, which results in inhibitory effects against hepatitis C virus [95], enterovirus [89] and herpes simplex virus replication and release

Table 2
Experimental evidence on the efficacy of DHCR7 or DHCR24 inhibition in pathogen infections.

Target enzyme	Inhibitory agent	Pathogen[1,2]	Experimental model[3]	References
DHCR7	AY9944	HIV-1 (VI, +ssRNA-RT)	Human PBM cells	[91]
	AY9944; siRNA	HCV (IV, +ssRNA)	HuH7 cells	https://pubmed.ncbi.nlm.nih.gov/22480142/ [133]
	siRNA	Astrovirus (IV, +ssRNA)	CaCo2 cells	https://pubmed.ncbi.nlm.nih.gov/26246569/ [134]
	AY9944; siRNA	ZIKV (IV, +ssRNA); VSV, SeV, and H1N1 (V, −ssRNA); EMCV (III, dsRNA); HSV (I, dsDNA)	Mouse macrophages; A549 cells; mice	https://pubmed.ncbi.nlm.nih.gov/31882361/ [92]
	AY9944	VSV (V, −ssRNA)	Neuro2a cells	https://pubmed.ncbi.nlm.nih.gov/36407960/ [93]
	AY9944; siRNA	EV-A71 and other enteroviruses (IV, +ssRNA)	HCT-8, RD, and Vero cells; mice	https://pubmed.ncbi.nlm.nih.gov/36528172/ [135]
	CoVR-MV (biomimetic antiviral nanobiologic)	SARS- and MERS-CoV (IV, +ssRNA)	Mouse macrophages; Syrian hamster	https://pubmed.ncbi.nlm.nih.gov/37096860/ [90]
	AY9944; shRNA	ZIKV (IV, +ssRNA)	U251 and Vero cells	https://www.sciencedirect.com/science/article/pii/S1995820X22001572 [94]
	AY9944; siRNA DHCR7$^{-/-}$	PRV (I, dsDNA)	HEK-293T, PK-15, Neuro2a, and Vero cells; mice	https://www.sciencedirect.com/science/article/abs/pii/S0378113524000221?via%3Dihub [119]
	AY9944	Sepsis (mainly of pulmonary and urinary origin)	Zebrafish	https://pubmed.ncbi.nlm.nih.gov/37332366/ [101]
DHCR24	U18666A; siRNA	HCV (IV, +ssRNA)	HepG2 and HuH-7; mice	https://pubmed.ncbi.nlm.nih.gov/21184787/ [95]
	U18666A	Prion	Neuronal cell lines	[74,75]
	DHCR24$^{-/-}$	HSV (I, dsDNA)	Mouse emibryonic fibroblasts	https://pubmed.ncbi.nlm.nih.gov/28446672/ [96]
	U18666A; siRNA	BVDV (IV, +ssRNA)	MDBK	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9769396/ [136]
	U18666A	EV-A71 (IV, +ssRNA)	HCT-8 cells	https://pubmed.ncbi.nlm.nih.gov/36528172/ [135]
	U18666A; siRNA; DHCR24$^{-/-}$	HSV (I, dsDNA); NDV and VSV (V, −ssRNA)	HepG2, HLCZ01, and HuH7 cells; mice	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10734464/ [97]

(1) Virus abbreviations and names: BVDV, bovine viral diarrhea virus; EMCV, encephalomyocarditis virus; EV-A71, non-polio enterovirus A71; HCV, hepatitis C virus; HSV, herpes simplex virus; H1N1, subtype of influenza A virus; MERS-CoV, middle east respiratory syndrome coronavirus; NDV, Newcastle disease virus; PRV, pseudorabies virus; SARS-CoV, severe acute respiratory syndrome coronavirus; VSV, vesicular stomatitis virus; SeV, sendai virus; ZIKV, zika virus.
(2) The Baltimore classification of viruses is provided in parenthesis.
(3) Cells abbreviations and names: A549, human lung adenocarcinoma; CaCo-2, human colorectal adenocarcinoma; HCT-8, human colon cancer; HEK-293T, human embryonic kidney; HepG2, HLCZ01, and HuH7, human hepatocellular carcinoma; MDBK, Madin-Darby bovine kidney; Neuro-2a, mouse neuroblast; PBM, peripheral blood mononuclear; PK-15, porcine kidney; RD, human embryonic malignant rhabdomyoma; U251, human malignant glioblastoma; Vero, normal african green monkey kidney epithelial.

of viral particles [96,97], showing that also DHCR24 may serve as an anti-microbial and immunoregulatory target. Altogether, these data suggest that the acute perturbation in the pool size of synthesized cholesterol triggers beneficial outcomes against infections. This proposed mechanism is sustained by the fact that the activity of AEBS components has been associated with M2 macrophage polarization [98–100] and the worsening of bacterial infections outcomes [101], in agreement with the reciprocal observation that SERMs polarize macrophages towards an inflammatory phenotype [53,54].

4. Mechanistic details of macrophage immune activation by AEBS inhibition

Under resting conditions, macrophages actively and tightly control intracellular cholesterol levels by finely tuning its uptake, storage and efflux, as well as utilization in cellular pathways, such as membrane formation or signal transduction processes, whereas during infections, the intracellular levels of cholesterol are reduced as a result of the increased expression of genes related with cholesterol homeostasis [102], a mechanism that is also triggered by SERMs through their effects on cholesterol accumulation in late *endo*-lysosomes and subsequent activation of transcriptional regulators of cholesterol metabolism [103,104]. In the course of infections, such metabolic adaptation limits cholesterol availability at the expense of the anabolic requirements of microbial cell proliferation [68,105] and triggers macrophage immune responses [30,106]. Aside from this knowledge, the molecular details underlying the immune and metabolic reprogramming induced by AEBS inhibition have only started to be elucidated and involve pre-genomic sensors and transcriptional regulators (see Fig. 2), as discussed below.

4.1. Acute pre-genomic effectors

TLRs. Local changes in cholesterol composition at intracellular membranes can also influence the subcellular distribution and function of receptors or adaptor systems that are key players for inflammation and other functions of innate immune cells. A classic example is represented by Toll-like receptors (TLRs) which, after binding specific

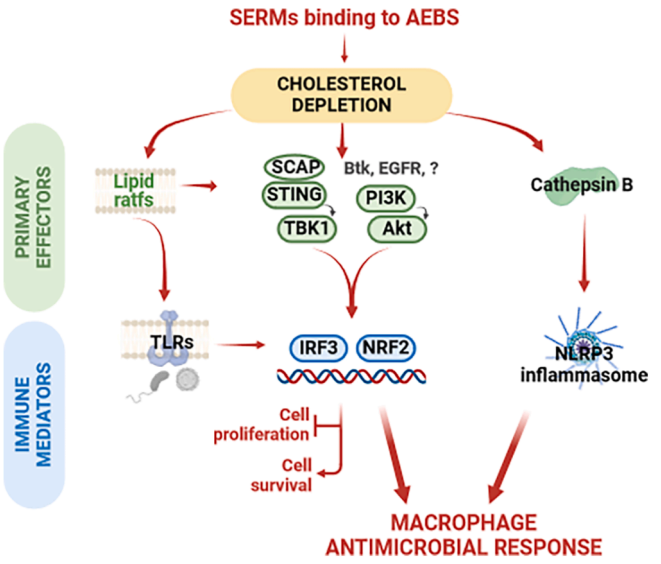

Fig. 2. Molecular details of the SERMs-AEBS immunomodulatory axis. Simplified schematic for AEBS-mediated pre-genomic and transcriptional targets of SERMs, by which these drugs regulate macrophage anti-infective performance. Alterations in cholesterol/lipid composition at cell membranes, induced by SERMs binding to AEBS, induce lipid raft changes that regulate TLR responses and activate cholesterol sensors/effectors that include the SCAP/STING/TBK1 and PI3K/Akt pathways, possibly linked with Btk or EGFR, which in turn induce IRF3 and NRF2 transcriptional programs and NLRP3 inflammasome signalling cascades. Together with antiproliferative and pro-survival effects, these immune mediators improve the macrophage antiviral and inflammatory responses. Created with BioRender.com.

microbial molecules, aggregate with signaling effectors at membrane lipid rafts and activate signal transduction pathways that lead to regulation of immune and antimicrobic factors [107]. Cholesterol depletion following treatment of macrophages with natural compounds has been shown to inhibit TLR translocation and to reduce inflammatory responses [108,109]. Notably, raloxifene and tamoxifen were reported to significantly increase the production of the Tumor Necrosis Factor α (TNFα) and Interleukin-1β (IL-1β), primary drivers of inflammation, in the presence of the TLR4 agonist, LPS [53,54], suggesting that AEBS inhibition and the reorganization of membrane components may underlie the activity of SERMs on TLR function and inflammatory processes.

NLRP3 inflammasome. Membrane cholesterol composition also impacts the NLR family pyrin domain containing 3 (NLRP3) inflammasome, a multiprotein complex that activates proteases, like caspase-1, to produce mature forms of the proinflammatory cytokines IL-1β and IL-18. Formation of the NLRP3 inflammasome complex in the endoplasmic reticulum requires the recruitment of NLRP3 partners that reside in different subcellular sites and merge with NLRP3 along cell activation. Key examples are the lysosomal enzyme cathepsin B and the purinergic P2X7 receptor (P2X7R), which operates within membrane lipid rafts. These proteins were show to transiently interact with and activate NLRP3 in selected macrophage subtypes and experimental conditions [110–113]. Cholesterol depletion or lysosome destabilization, can lead to the activation of cathepsin B and P2X7R [114–116]. Interestingly, recent work showed that SERMs prompt cathepsin B [unpublished results], NLPR3 inflammasome and caspase-1 activation in macrophages [53,54], further sustaining the connection between SERMs modulation of cholesterol biosynthesis and macrophage inflammatory response. The mechanisms that link AEBS inhibition with the induction of these immediate immune responses are still not clear. The accumulation of SERMs in the *endo*-lysosomal compartment, due to their cationic amphiphilic nature, could induce structural alterations similarly with other molecular species that were shown to trigger structural rearrangements and induce novel contacts sites within organelles and signaling pathways [117,118]. By modifying lysosomal homeostasis, SERMs would be able to bind to AEBS in the endoplasmic reticulum, thereby changing cholesterol composition and inducing inflammasome activation and immune responses.

4.2. *Transcriptional immune mediators*

Cholesterol depletion has been associated with the activation of key immune transcription factors, namely interferon regulatory factor 3 (IRF3), which is implicated in type I interferons (IFN-I) and interferon-stimulated gene expression, and nuclear factor erythroid 2-related factor 2 (NRF2), which is involved in the regulation of inflammatory, antioxidant and survival gene expression.

IRF3. Mechanistic studies showed that limiting cholesterol biosynthesis, through the use of tamoxifen [92] as well as pharmacological or genetic inhibitors of DHCR7 [89,90,92,119] and DHCR24 [97], enhances IRF3 activity, which results in increased IFN-β production in macrophages infected by different viral species [92]. Correspondingly, the induction of DHCR7 or DHCR24 expression that is attained by certain viral infections represses the IRF3 and IFN-I cascade [94,120].

Being inactive in the cytoplasm under resting conditions, IRF3 is activated through its phosphorylation elicited by kinases, such as TANK-binding kinase 1 (TBK1) and the phosphoinositide 3-kinase(PI3K)- AKT serine/threonine kinase (Akt) pathway, that allow its nuclear migration and transcriptional activity [121]. In depth studies argued that the decline in cholesterol levels activates the stimulator of interferon genes (STING), an endoplasmic reticulum-localized protein that senses cholesterol reduction through cholesterol-binding motifs that are present in the protein sequence; the resulting conformational changes induce STING translocation to the Golgi apparatus, where it recruits and activates TBK1 kinase with the possible involvement of the scaffold adaptor protein, SREBP cleavage-activating protein (SCAP), leading to IRF3 activation (see Fig. 2) [122,123]. Such mechanisms are also normally implemented by macrophages as defense mechanisms against microbial infections [92,106].

This cholesterol-immunity transcriptional circuit has been also described for the transcription factor sterol regulatory element-binding protein 2 (SREBP2), a master transcriptional regulator of enzymes that regulate cholesterol trafficking. In macrophages, SREBP2 transcriptional activation by cholesterol restriction has been shown to require the intervention of SCAP, a chaperone protein that escorts SREBP2 from the endoplasmic reticulum towards a proteolytic apparatus that cleaves SREBP2 and favors its nuclear translocation [124]. While still explorative, all these studies helped in identifying the molecular mechanisms that translate the reduction in cholesterol biogenesis with the activation of specific IRF3 and SREBP2 transcriptional programs.

NRF2. This transcription factor strongly influences metabolic and inflammatory responses once it is able to migrate into the nucleus after the removal of an inhibitory complex [125]. Recently, tamoxifen and raloxifene were shown to increase NRF2 stability and transcriptional activity in parallel with the regulation of the production of immune mediators induced by TLR4 stimuli, in line with data on the NRF2-mediated protective effects of raloxifene in a model of endotoxemia [53,54,126]. Interestingly, NRF2 activation has been shown to enhance macrophage survival [127,128] and could represent a key mediator of the antiproliferative and pro-survival effects of SERMs in macrophages. Thus, it is hypothesized that SERMs act in a macrophage-specific manner by intensifying NRF2-mediated cell-intrinsic mechanisms that, on one side, limit cell proliferation and hyperinflammation and, on the other, fuel selected inflammatory responses and cell survival (see Fig. 2). As in the case of IRF3, also NRF2 induction by SERMs is still far from being elucidated. The PI3K/Akt pathway has been shown to activate NRF2 following SERMs treatment [53,54], by a mechanism that could be ascribed to the phosphorylation and inactivation of constitutive systems that typically repress NRF2 activity [125]. Cholesterol reduction in

macrophages has been reported to activate the PI3K/Akt axis [92], but the upstream cholesterol-sensors are still not defined. It has been shown that cholesterol depletion forms ordered membrane microdomains that activate the epidermal growth factor receptor (EGFR) and PI3K signaling pathways in fibroblasts [129]. Moreover, it has been shown that the endosomal re-localization of the Bruton's tyrosine kinase (Btk) allows this enzyme to activate the PI3K, NLRP3 and NRF2 signaling pathway in TLRs-stimulated macrophages [130,131]. Whether tyrosine kinases, adaptor chaperones or other proteins intervene in the PI3K-dependent activation of NRF2 by SERMs still needs to be determined.

5. Conclusions and future perspectives

Tamoxifen and other SERMs have shown promising activity against a broad range of important human pathogens and are indeed increasingly considered a potential and rapid way to achieve significant results in the treatment of infectious disease [26,27]. Furthermore, as a class of pharmaceuticals SERMs are generally considered safe and well tolerated, particularly when used in the short-term, which makes them suitable for the repurposing application as modulators of the immune system.

Among the multiple off-targets of SERMs, AEBS multiprotein complex appears to be the most conceivable and exploitable mediator of their immunomodulatory activity, being a high affinity and efficient tuner of macrophage cholesterol metabolism and immune homeostasis.

With the aim to examine the effect of SERMs on the AEBS-macrophage interplay and its significance in antimicrobial therapy, we collected evidence from the literature and results from our laboratory that indicate the followings: 1) AEBS-related mechanisms are critical in infection progression or chemotherapy; 2) SERMs improve the immune activity of macrophages through this ER-unrelated mechanism that implicates restriction of cholesterol biosynthesis, with the additional opportunities to efficiently suppress pathogen invasion and to moderate harmful host responses; 3) downstream effectors of SERM-mediated AEBS inhibition include immune complexes, receptors and transcription factors (Fig. 2).

While still in its infancy, studies on the molecular and biological mechanism of SERMs action via AEBS inhibition will help in developing novel candidate targets of immunopharmacological attempts. We hope to stimulate further research in this field to delineate the precise role of the SERM-AEBS-cholesterol axis in immune reactivity to infections and improve the prospects for survival of the host cell.

CRediT authorship contribution statement

Chiara Sfogliarini: Writing – review & editing, Writing – original draft, Conceptualization. **Lien Hong Tran:** Writing – review & editing. **Candida Maria Cesta:** Writing – review & editing. **Marcello Allegretti:** Writing – review & editing. **Massimo Locati:** Writing – review & editing, Writing – original draft, Conceptualization. **Elisabetta Vegeto:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maria Candida Cesta and Marcello Allegretti are employees of Dompé farmaceutici S.p.A. All other authors declare no competing interests.

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