Seladin-1 as a target of estrogen receptor activation in the brain: a new gene for a rather old story?

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REVIEW ARTICLE

Seladin-1 as a target of estrogen receptor activation in the brain: A new gene for a rather old story?

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ABSTRACT. Experimental evidence indicates that estrogen exerts neuroprotective effects. According to the fact that Alzheimer's disease (AD) is more common in post-menopausal women, estrogen treatment has been proposed. However, the beneficial effect of estrogen or selective estrogen receptor modulators (SERMs) in preventing or treating AD is a controversial issue, which will be summarized in this review. Recently, a novel gene, named selective AD indicator-1 (*seladin-1*), has been isolated and found to be down-regulated in brain regions affected by AD. *Seladin-1*, which is considered the human homolog of the plant protein DIMINUTO/DWARF1, confers protection against β-amyloid-mediated toxicity and from oxidative stress and is an effective inhibitor of caspase 3 activity, a key mediator of apoptosis. This review will present the up-to-date findings regarding *seladin-1* and DIMINUTO/DWARF1. In addition, the possibility that *seladin-1* may be a downstream effector of estrogen receptor activation in the brain, based on our recent experimental findings using a human fetal neuronal model, will be addressed.

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INTRODUCTION

Epidemiological data, together with experimental and clinical evidence, appear to support a neuroprotective role of estrogen and hormonal therapy in Alzheimer's disease (AD), the most prevalent neurodegenerative disorder in the elderly, has been suggested. However, there is no general consensus on this point, which will be briefly summarized in this review. As a matter of fact, this story is far from being concluded. A debated question concerns the factors which act as downstream mediators of estrogen receptor activation in the brain. The recent identification of the selective AD indicator-1 (*seladin-1*) gene and the finding that it protects the brain from toxic insults led us to hypothesize that this gene might represent the link between estrogen and neuroprotection. *Seladin-1* shares a high degree of sequence homology and some biological functions with a previously identified plant enzyme, named DIMINUTO/DWARF1, which is involved in the biosynthesis of brassinosteroids (BRs), an important class of plant hormones. This review will present the current knowledge on *seladin-1* and on its plant homolog DIMINUTO/DWARF1. Furthermore, the possible role of *seladin-1* as a target of estrogen receptor activation in the brain, based on our recent experimental findings, will be addressed.

ESTROGEN/SERMS AND NEUROPROTECTION: EXPERIMENTAL AND CLINICAL EVIDENCE

This topic has been extensively reviewed by many authors and the current experimental and clinical knowledge will be briefly reported in this review. It is well known, based on *in vitro* evidence, that estrogen exerts neurotrophic and neuroprotective effects by stimulating the expression of neurotrophins and cell-survival factors, enhancing synaptic plasticity and acting as an antioxidant factor (1-3). Besides the hypothalamus, which is the traditional site of estrogen action in the brain, both the estrogen receptor

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α (ERα) and β (ERβ) have been found, for instance, in the neocortex and in the hippocampus, two brain areas highly involved in AD (2). AD, which is the most prevalent form of late-life mental failure in humans, is characterized by a progressive impairment of cognitive functions, such as memory and language. The histopathological hallmark of AD is represented by the accumulation of extracellular β-amyloid plaques and intracellular neurofibrillary tangles, which are responsible for a complex inflammatory response leading to neuronal degeneration and cell death (4). Unfortunately, there is still no reliable way of predicting the onset of the disease and of effectively curing it. The fact that experimental evidence advocates a favorable estrogen effect in neurons, together with the knowledge that AD is more common in women and decreased estrogen levels after menopause are a risk factor for the disease (5), led to the suggestion that estrogen therapy might be beneficial. To date, despite the lack of general consensus, several studies have indicated that estrogen treatment may decrease the risk or delay the onset of AD in postmenopausal women (6). Conversely, the recent data from the Women's Health Initiative Memory Study trial and from the trial of estrogen treatment for AD showed that hormone replacement therapy (HRT) has no benefit. However, it has to be remembered that different factors may determine the efficacy of estrogen or HRT, such as age, menopausal status, pre-existing risk factors (i.e. smoking, apolipoprotein E genotype) (7-9). In particular, there seems to be a critical time for estrogen treatment. In fact, early and prolonged therapy has been found to produce the maximum benefit in terms of reduced risk for AD (10, 11). In addition, estrogen therapy is not the same as HRT, and the type of progestogen used determines the outcome of the therapeutic intervention (9).

A debated question is whether the protection conferred by classical nuclear estrogen receptors is mediated by the α or the β subtype or by both. Although the results from ERα knockout (ERKO) and ERβ knockout (βERKO) mice are somewhat controversial, it is worth noting that, whereas 17βestradiol ($17\beta E_2$) exerted a protective effect in the brain of ovariectomized βERKO mice, it did not in ERKO mice, thus suggesting a critical role for ERα in neuroprotection (12). Furthermore, it is worth mentioning that decreased expression of ERα in hippocampal neurons of AD patients has been observed (13). However, a possible role for ERβ in neuroprotection has been postulated, based on the evidence that βERKO mice undergo increased neuronal loss throughout life compared with wild-type controls (14). In addition to classical nuclear ERs, more recent findings suggest that the brain contains a plethora of ERs. For instance, a third member of the ER family, ERγ, has been identified (15). Furthermore, a variety of nuclear, cytoplasmic and plasma membrane ERs has been described in the brain, including for instance G protein-coupled receptors, a novel membrane-associated ER-X and a truncated ERα variant (16).

Less is known about the neuroprotective role of the selective estrogen receptor modulators (SERMs). Nonetheless, a neuroprotective effect of tamoxifen (TMX) and raloxifene (Ral) has been observed (17), and a beneficial role of TMX and Ral against β-amyloid toxicity has been demonstrated in rat neurons (18, 19). In addition to neuroprotective effects, there is increasing evidence that SERMs may also be neurotrophic, by increasing for instance synaptic density and stimulating neurite outgrowth (17). Data regarding the clinical use of SERMs in AD are very limited, so far. However, from the Multiple Outcomes of Raloxifene Evaluation trial, in which more than 7000 women with osteoporosis were assigned to receive Ral (60 or 120 mg) or placebo daily for three years, a trend toward less cognitive decline in the Ral group on tests of verbal memory and attention was observed (20).

In summary, the basic science strongly supports a neuroprotective role of estrogen/SERMs. Although currently there is no clear cut evidence that these molecules can decrease the risk or ameliorate the clinical course of AD, it is conceivable that there might be a proper space for a hormonal-based intervention in this disease.

THE IDENTIFICATION AND CHARACTERIZATION OF SELADIN-1

In 2000, Greeve et al. (21) used a differential mRNA display approach to identify genes that were differentially expressed in selective vulnerable brain regions in AD. In fact, although intracellular neurofibrillary tangles and extracellular and perivascular deposits of β-amyloid, the histopathological hallmarks of AD, are evenly distributed throughout the brain, substantial loss of neurons and synapses occurs only in selective brain regions, such as the hippocampus, the amygdala, the inferior temporal cortex and the enthorhinal cortex (4). Conversely, neurons populating other brain regions, such as the frontal, the parietal and the occipital cortex, are protected from degeneration. Among the >30 genes differentially expressed in AD vulnerable brain regions *vs* unaffected areas, Greeve et al. identified a novel cDNA with a markedly reduced expression in the inferior temporal cortex of AD patients compared to the frontal cortex, obtained shortly *post mortem*. Conversely, this cDNA was evenly expressed in the brain of unaffected individu-

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als. This gene was named *seladin-1* from selective Alzheimer's disease Indicator-1 and its full-length cDNA was cloned from a human brain cDNA library (GenBank accession number AF261758). The *seladin-1* gene spans 46.4 kb, maps to chromosome 1p31.1-p33, and comprises nine exons and eight introns; it encodes an open reading frame of 516 amino acid residues. To localize the cellular distribution of *seladin-1*, human H4 neuroglioma cells were transfected with a *seladin-1*-green fluorescent protein (GFP) fusion construct. *Seladin-1* appeared to be mainly located in the endoplasmic reticulum and, although to a lesser extent, in the Golgi apparatus.

With regard to its biological effects, *seladin-1* was found to confer resistance against β-amyloid and oxidative stress-induced apoptosis and to effectively inhibit the activation of caspase 3, a key mediator of the apoptotic process. Interestingly, in PC12 cells that were selected for resistance against β-amyloid toxicity, the level of expression of *seladin-1* was remarkably high. A subsequent study demonstrated that the down-regulation of *seladin-1* expression in vulnerable AD brain areas is paralleled by an increase in the amount of hyperhosphorylated tau, a protein component of neurofibrillary tangles (22).

Apart from the brain, *seladin-1* mRNA has also been detected in many different human organs and the highest levels of expression have been found in the adrenal gland, the liver, the lung and the prostate. In the adrenal cortex, *seladin-1* expression is stimulated by ACTH *via* the cAMP-dependent pathway: accordingly, in cortisol-secreting adenomas *seladin-1* appeared to be overexpressed compared to the atrophic adjacent adrenal tissue (23). In a subsequent study, we observed that the expression level of *seladin-1* in adrenal carcinomas is significantly lower than in the normal adrenal and in adrenal benign cortisol- or aldosterone-secreting adenomas (24). These results are in agreement with the very recent finding that *seladin-1,* upon binding to p53, may also have a tumor suppressor role, involved in cellular response to Ras/p53-mediated oncogenic signaling (25).

An important step forward in discovering the biological properties of *seladin-1* was represented by the demonstration that this protein also has a specific enzymatic activity, which was found to be markedly reduced in desmosterolosis, a rare autosomal recessive disorder characterized by multiple congenital anomalies (26). In fact, patients with desmosterolosis have elevated plasma levels of the cholesterol precursor desmosterol (Fig. 1), and this abnormality suggested a deficiency of the enzyme 3β-hydroxysterol Δ^{24}-reductase (DHCR24), which catalyzes the reduction of the Δ^{24} double bond in desmosterol to produce cholesterol. Waterham et al. (27) were able to identify the human DHCR24 cDNA, which appeared identical to *seladin-1*. DHCR24 activity was confirmed *in vitro* by enzymatic assay following heterologous expression of the DHCR24 cDNA in *Saccharomyces cerevisiae*. Conversely, in constructs containing mutant DHCR24 alleles from patients with desmosterolosis the conversion from desmosterol into cholesterol was nil or markedly reduced.

Desmosterolosis belongs to a group of several inherited disorders, linked to enzyme defects in the cholesterol biosynthetic pathway at the post-squalene level, which have been described in recent years (Fig. 1) (28). These genetic diseases are characterized by major developmental malformations and in most cases determine severe neuro-psycological alterations, suggesting an important role for cholesterol in brain homeostasis. The role of cholesterol in facilitating the onset and progression of AD is a debated and unsolved question. In fact, on one hand cholesterol may be viewed as a toxic factor. Elevated cholesterol levels increase β-amyloid formation in *in vitro* systems and in most animal models of AD (29, 30). The identification of the ε4 allelic variant of the apolipoprotein E as a major genetic risk factor for AD is also consistent with a role for cholesterol in the pathogenesis of this disease. Accordingly, epidemiological studies suggest that statin therapy may provide protection against AD (31), although the clinical benefit of statins might also be due to their cholesterol-independent effects on cerebral circulation and inflammation (31). On the other hand, it has to be considered that the central nervous system (CNS) is a very unique structure with regard to cholesterol metabolism: in fact, although the CNS accounts for only 2% of the entire body mass, it contains about 25% of the total amount of unesterified cholesterol in the entire body. In addition, most of the CNS cholesterol is produced *via* local *de novo* synthesis. Keeping this in mind, it is not surprising that several studies pointed out the fact that the intracellular content of cholesterol, particularly the amount contained in the cell membrane, should be addressed much more than its plasma levels (29). In this new scenario, an appropriate amount of membrane cholesterol would create a barrier against toxic insults, whereas a cholesterol-depleted membrane would ease the interaction with toxic factors such as β-amyloid, which may generate for instance an anomalous number of calcium channels leading to the accumulation of toxic levels of calcium (32). Interestingly, in AD patients impaired or lack of 3-hydroxy-3-methylglutaryl-CoA reductase activity in the affected brain regions has been reported. Undoubtedly, the reduced expression of *seladin-1* in AD vulnerable regions is in keeping with the "membrane integrity" theory.

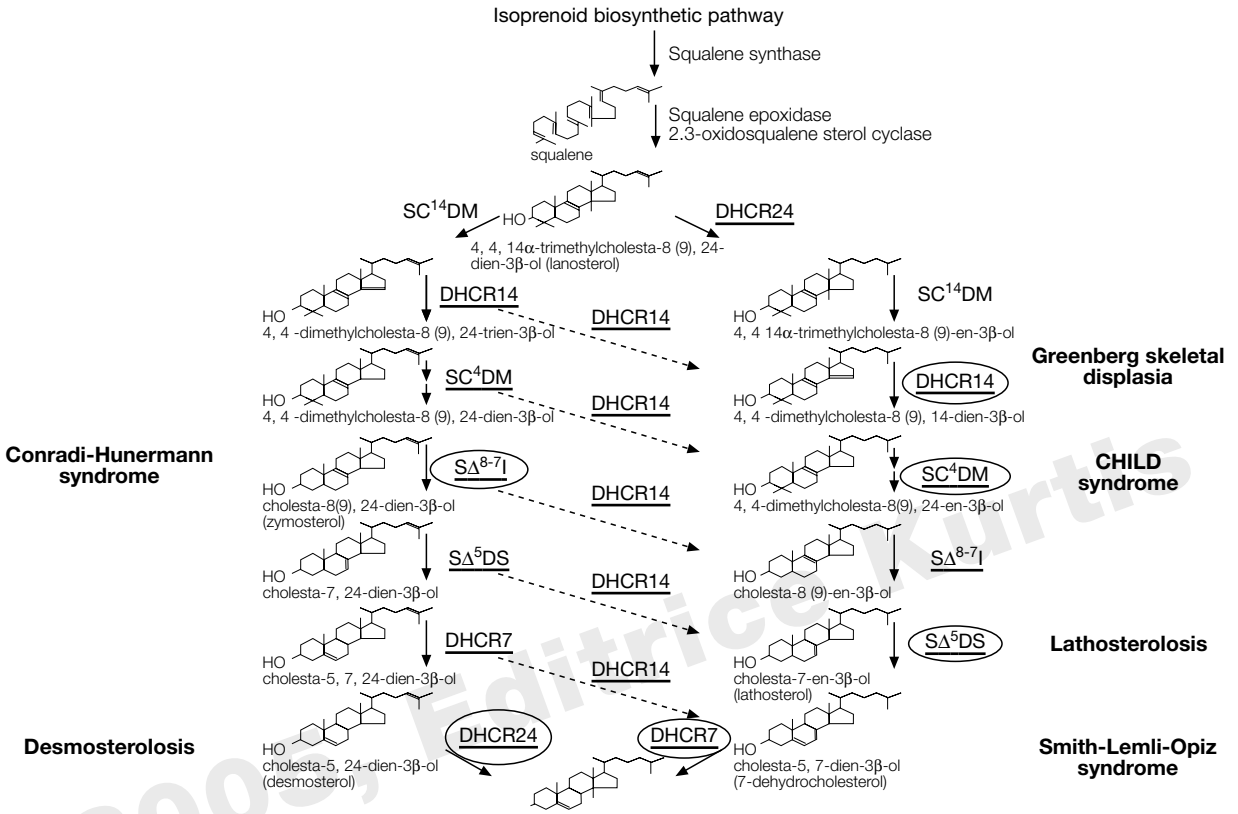

Fig. 1 - *Cholesterol biosynthesis from squalene. Deficiencies of the circled enzymes are responsible for different inherited disorders of cholesterol biosynthesis. DHCR24: 3β-hydroxysterol Δ^{24}-reductase (Desmosterolosis); $SC_{14}DM$: 3β-hydroxysterol C_{14} demethylase; DHCR14: 3β-hydroxysterol Δ^{14}-reductase (Greenberg skeletal dysplasia); SC4DM: 3β-hydroxysterol C_4 demethylase complex (including a 3β-hydroxysteroid dehydrogenase defective in CHILD syndrome); $S\Delta^{8-7}I$, 3β-hydroxysterol Δ^8-Δ^7 isomerase (Conradi-Hunermann syndrome); $S\Delta^5DS$: 3β-hydroxysterol Δ^5-desaturase (lathosterolosis); DHCR7: 3β-hydroxysterol Δ^7-reductase (Smith-Lemli-Opiz syndrome). Modified from (28).*

Recently, mice with a targeted disruption of the DHCR24 gene have been generated (33). As expected, plasma and tissues of DHCR24-/- mice contained virtually no cholesterol, whereas desmosterol accumulation was observed. These animals were around 25% smaller in size than DHCR24+/+ and DHCR24+/- littermates at birth and survived to adulthood, although with poor growth features. Histologically, all organs showed a normal structure with the exception of the testes, in which germ cell degeneration was observed. Both males and females were infertile. Admittedly, the relatively mild phenotype of DHCR24-/- is in overt contrast with the severe abnormalities observed in patients with desmosterolosis. It has been speculated that this discrepancy may be due to the fact that maternal cholesterol is not available during human embryogenesis, as it is in mice. Alternatively, desmosterol, which has been shown to replace cholesterol in mouse fibroblasts without toxic effects (34), might act as a "cholesterol-mimetic" steroid in DHCR24-/- animals.

Another interesting aspect related to the identification of the *seladin-1* gene is represented by the high degree of sequence homology and by the similar enzymatic activity shared with the plant enzyme DIMINUTO-DWARF1, described in Arabidopsis thaliana.

THE PLANT DIMINUTO/DWARF1 GENE

DIMINUTO/DWARF1 is a gene encoding for an enzyme involved in the biosynthetic pathway of the most active BR, brassinolide (35) (Fig. 2). BRs are a class of sterol plant hormones that can be considered as the counterpart of animal steroid hormones. They have been shown to regulate gene expression, stimulate cell division and differentiation, and modulate reproductive biology (36). BRs also mediate growth response unique to plants, including the promotion of cell elongation in the presence of a complex cell wall and the multiple developmental responses to darkness and light.

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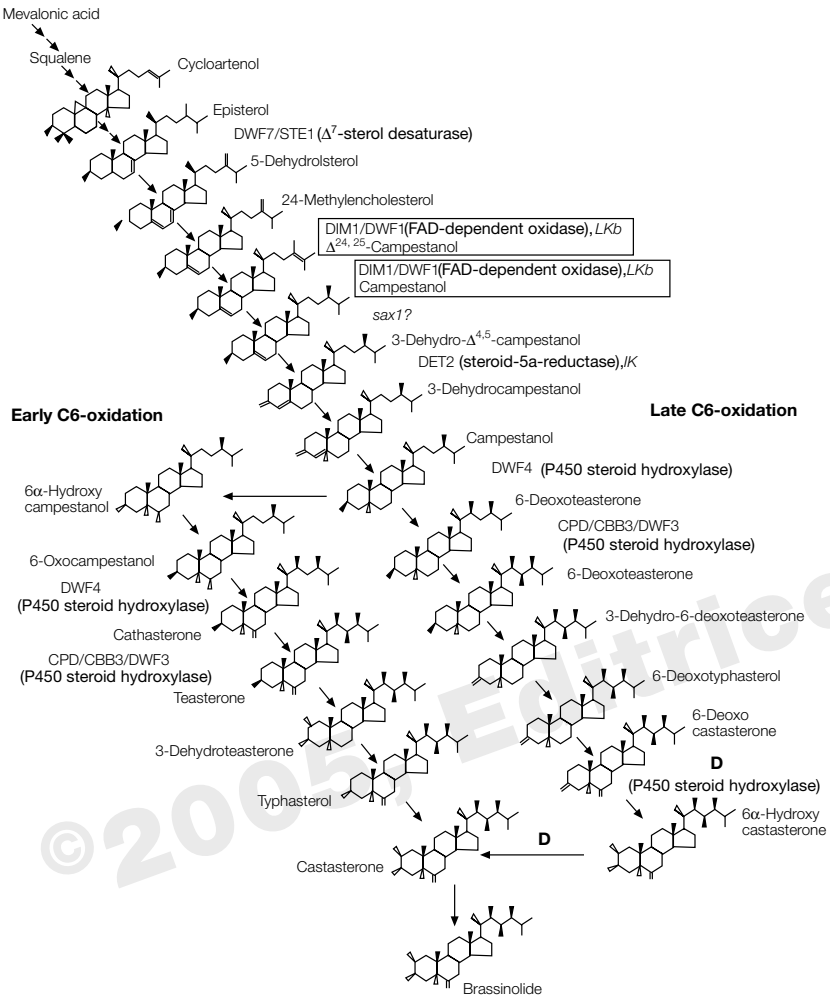

Fig. 2 - *Biosynthetic pathway of brassinolide. The plant homolog of* seladin-1, *DIMINUTO/DWARF1(DIM1/DWF1) is circled. Modified from (35).*

The function of DIMINUTO/DWARF1 was identified analyzing the dim mutant of Arabidopsis thaliana, which shows a severe dwarf phenotype with reduced fertility (37). The mutant phenotype could be rescued by the addition of exogenous brassinolide or brassinolide precursors, indicating a role in BR biosynthesis. The specific enzymatic step in which DIMINUTO/DWARF1 is involved was elucidated by the analysis of endogenous sterols in the mutant. In fact, the dim mutant accumulates 24-methylencolhesterol and is deficient in campesterol, a key precursor of brassinolide. Feeding experiments with the deuterated precursor established that this protein catalyzes the isomerization of 24-methylencolhesterol to 24-methyldesmosterol and successively the reduction of the $\Delta^{24\text{-}25}$ double bond to yield campesterol. The same reactions result in the synthesis of sitosterol from isofucosterol. Transient expression of the fusion protein DIMINUTO/DWARF1 with GFP strongly suggests that DIMINUTO/DWARF1 is an integral membrane protein probably associated with the endoplasmic reticulum.

DIMINUTO/DWARF1 is therefore responsible for the synthesis of the main plant membrane sterol lipids sitosterol and campesterol, the latter being also the key precursor of the steroid-like hormones BRs. Sterols are used by many organisms both as modulators of membrane elasticity and as precursors for the synthesis of steroid hormones. Animals mainly synthesize cholesterol, which serves as a precursor of steroid hormones after the cleavage of the alkyl side chain, and as a membrane sterol lipid. Plants use campesterol as a hormone precursor and both sitosterol and campesterol as membrane lipids.

The plant protein DIMINUTO/DWARF1 and *seladin-1* share a similar function as sterol biosynthetic

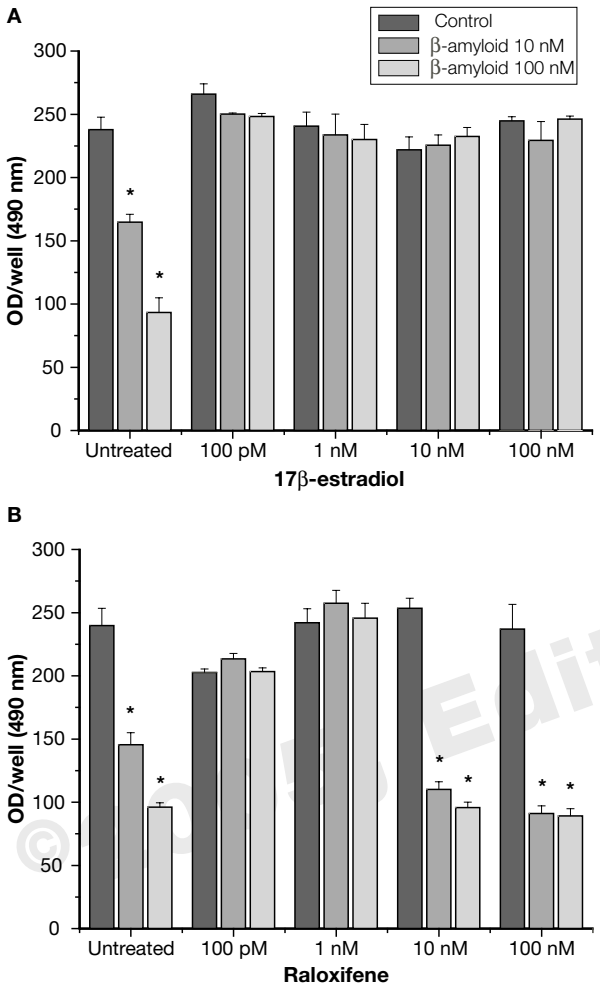

Fig. 3 - *Effect of different concentrations of 17β estradiol ($17\beta E_2$) (A), or raloxifene (B) on β-amyloid (10 and 100 nM) toxicity, as assessed by MTS assay (Promega Corp., Madison, WI). *=p<0.05 vs untreated control cells.*

enzymes. Unlike humans, the defect of this function does not influence plant embryogenesis, that seems to be influenced by upstream precursors of 24-methylencholesterol (24-methylenlophenol) (38). Moreover, the disruption of the DIMINUTO/DWARF1 gene seems to expand the life span of the dim mutant and this phenomenon is probably associated to the reduced fertility of the plant (39). Interestingly, Arabidopsis with a mutation in one of the gene homologues of 3-hydroxy-3-methylglutaryl-CoA reductase (40), the first step in the isoprenoid biosynthesis, shows an early senescence indicating that the roles of plant sterols are probably more diversified and structure-dependent than in animals where cholesterol seems to perform multiple functions. Nevertheless, unraveling the biochemistry and the biological functions of sterols in plants may represent a fundamental step in the comprehension of the involvement of this class of lipids in human physiology and pathology. What is noteworthy is that plant-derived sterols have been found in mammal brain (41), opening a new window on their possible therapeutic use in human diseases. With regard to this point, the identification of the *seladin-1* gene as the human homolog of the plant DIMINUTO/DWARF1 gene and the discovery of its implication in AD, which represents a scientific and medical problem but also a profound social and economic burden, constitutes an important advancement in understanding the biology of neuronal cells.

SELADIN-1: A NEW EFFECTOR OF ER-MEDIATED NEUROPROTECTION?

Because of the parallelism between some of the biological properties of *seladin-1* and the effects of estrogen and SERMs in neurons, we hypothesized that *seladin-1* might be targeted as a downstream effector of the activation of estrogen receptors in the brain. As mentioned previously, there is experimental evidence that estrogen and SERMs may confer neuroprotection. However, in most of the studies which have been performed so far, animal models have been used. In other cases human cells, transformed or of neoplastic origin, have been used. We had the opportunity to study the neuroprotective effects of estrogen/SERMs using a unique human cell model. This model is represented by GnRH-secreting neuroblast long-term cell cultures from human fetal (8-12 weeks of gestational age) olfactory epithelium. These cells, named FNC, were

Fig. 4 - *TUNEL analysis of β-amyloid-induced apoptosis. A) control FNC cells; B) cells exposed to 100 nM β-amyloid. The arrows indicate fragmented nuclei derived from clumps of cells; C) cells pretreated with 100 pM 17β estradiol ($17\beta E_2$) before β-amyloid exposure (100 nM).*

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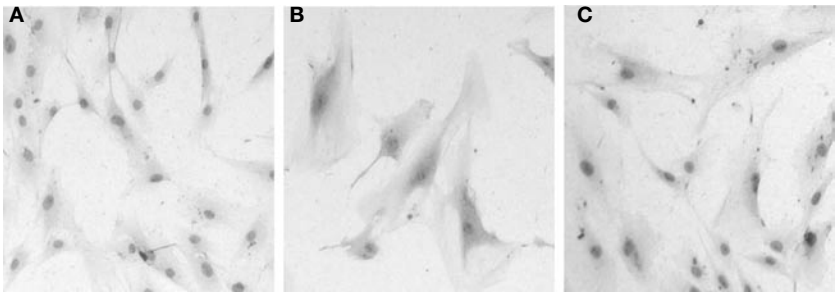

Fig 5 - *Immunocytochemical detection of cleaved caspase 3. A) control FNC cells; B) immunostained cells, with a variable degree of positivity, after exposure to 100 nM β-amyloid; C) cells pretreated with 100 pM 17β estradiol (17βE_2) before β-amyloid exposure (100 nM).*

established, cloned and propagated previously by Vannelli et al. (42) at the Department of Anatomy, Histology, and Forensic Medicine of the University of Florence. They showed unique features, because they stained positively for neuronal and olfactory markers, such as neuron specific-enolase and vimentin, which target maturing olfactory receptor neurons, and neural cell adhesion molecule and olfactory marker protein, which appear at a relatively later stage during differentiation (42). FNC cells are electrically excitable and following exposure to a number of different aromatic chemicals showed a specific increase in intracellular cAMP, indicating some degree of functional maturity. Thus, FNC cells appear to originate from the stem cell compartment that generates mature olfactory receptor neurons. In addition, they express both ERα and ERβ (43). Therefore, they represented an appropriate, and most importantly human, *in vitro* model, that could be of help in providing further information on the role of estrogen in neurons, and in answering the question whether *seladin-1* may be an effector of ERs activation.

We first evaluated the role of 17βE_2 on FNC survival after β-amyloid exposure. We observed that, whereas in the absence of estrogen pre-incubation β-amyloid (10 and 100 nM for 18 h) significantly and dose-dependently reduced cell viability (Fig. 3A), the pre-treatment with 17βE_2 (100 pM-100 nM for 72 h) effectively counteracted the effect of β-amyloid (44). Noticeably, this effect was independent of increased cell proliferation rate, which was not changed by exposure to estrogen for 72 h. Virtually superimposable data were obtained using the SERM TMX (100 pM-100 nM) (data not shown), whereas partially different results were observed using Ral. In fact, cell viability was preserved at low concentrations of Ral (100 pM and 1 nM). Conversely, 10 and 100 nM Ral did not protect against β-amyloid-induced toxicity (Fig. 3B). Similarly to estrogen, neither TMX nor Ral had any effect on cell proliferation rate. A protective effect of 17βE_2 against oxidative stress (200 μM H_2O_2) was also observed: in fact, the negative effect of H_2O_2 on cell viability was prevented by pre-exposure to 100 pM-100 nM 17βE_2 (not shown).

Next, in order to determine whether the protective action of estrogen in FNC cells was associated to an effect on apotosis, the amount of apoptotic cells following β-amyloid exposure, in the absence or in the presence of 17βE_2, was evaluated. TUNEL analysis clearly revealed that the pro-apoptotic effect of 100 nM β-amyloid was counteracted by estrogen (100 pM 17βE_2) (Fig. 4). Similarly, the activation of caspase 3, a key mediator of apoptosis, which was induced by β-amyloid, was prevented by estrogen pre-treatment (Fig. 5).

Finally, in order to answer the question whether estrogen and/or SERMs have an effect on *seladin-1* expression, we evaluated the amount of *seladin-1* mRNA in FNC cells, treated or not with 17βE_2, TMX

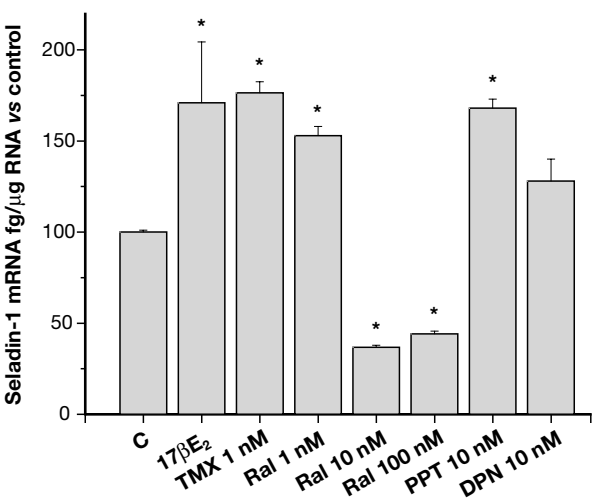

Fig. 6 - *Amount of* seladin-1 *mRNA, assessed by real-time RT-PCR, in untreated control FNC cells (C), in cells treated with 1 nM 17β estradiol (17βE_2) or tamoxifen (TMX), with raloxifene (Ral) (1-100 nM), with 10 nM propylpyzazole (PPT) or diarylpropionitrile (DPN). *=p<0.05 vs control cells (C) value (112±2.26 fg/μg total RNA, mean±SE), considered as 100%.*

or Ral, by quantitative real-time RT-PCR based on TaqMan technologies. We found that FNC cells constitutively express *seladin-1* (112±2.26 fg/μg total RNA, mean+SE) (Fig. 6). Noticeably, 17βE_2 (10 pM-100 nM) significantly increased the amount of *seladin-1* mRNA. 1 nM TMX or Ral determined a similar increase of *seladin-1* mRNA, compared to an equal concentration of 17βE_2. However, higher concentrations of Ral (10-100 nM) determined a marked reduction of *seladin-1* expression, in keeping with the lack of a neuroprotective effect. The effect of a selective ERα [propylpyrazole-triol, (PPT)] or a selective ERβ [diarylpropionitrile (DPN)] agonist was also tested. We found that PPT determined a significant increase in the amount of *seladin-1* mRNA at a concentration (10 nM) which has been reported to induce evident transcriptional activity (45), whereas DPN produced a weaker effect, thus indicating a predominant role of ERα in mediating the stimulatory effect of estrogen on *seladin-1* expression.

CONCLUSIONS

Our original results strongly support a role for ERs-mediated neuroprotection. A rather unique feature of our study is represented by the fact that the experimental data have been generated using human neuroblasts. In addition to 17βE_2, we also provided evidence that the SERMs TMX and Ral may act as estrogen agonists in the brain and highlight the possibility that SERMs may be beneficial in the treatment of neurodegenerative disorders such as AD.

Our study also addressed for the first time the role of *seladin-1* in mediating the effects of ERs activation in the brain. In the *in vitro* model we used we demonstrated that estrogen, TMX and Ral (the latter only at low concentrations) increase the expression level of *seladin-1*. Although a conclusive statement on the role of *seladin-1* as an effector of the neuroprotective role of estrogen cannot be advocated at present, our findings suggest that this might be a likely hypothesis. In fact, the marked parallelism between the concentrations of Ral which provide neuroprotection and the concentrations which stimulate *seladin-1* expression appears to be strongly in favor of this hypothesis. The fact that estrogen is able to stimulate *seladin-1* expression on one hand and to prevent β-amyloid-induced activation of caspase 3 on the other hand, further supports a role for *seladin-1* as a transcriptional target of estrogen. Nevertheless, additional studies, specifically designed to study for instance the effect of estrogen/SERMs upon silencing *seladin-1* expression, will be needed to further confirm the involvement of this gene in hormonal-mediated neuroprotection.

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