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Review

Statins and prostate cancer: Molecular and clinical aspects

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ABSTRACT

The field of the potential applications of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) beyond their unambiguous cardiovascular beneficial effects is steadily increasing. In this regard, statins have also been shown to possess anti-inflammatory, immunomodulatory, antioxidant and growth inhibitory properties. Regarding their role in carcinogenesis, both preclinical and clinical studies report conflicting results. Intriguingly, accumulating evidence suggests that statins may relate to decreased prostate cancer incidence and recurrence risk. However, data from clinical studies seem to be still weak and are confounded by several factors. Nonetheless, preclinical data suggest that statins might exert a chemopreventive role against prostate cancer by inhibiting the proliferation and inducing apoptosis of prostate cancer cells and also inhibiting angiogenesis, inflammation and metastasis. Cholesterol lowering as well as statin pleiotropy through inhibition of the synthesis of isoprenoids have both been implicated in their anticancer properties. In this review, we discuss the preclinical and clinical evidence supporting the preventive or potentially harmful effects of statins on prostate tumourigenesis and conclude that statins should not be recommended for the prevention of prostate cancer development or progression based on the current data.

1. Introduction

3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) constitute a low molecular weight drug family that is widely and efficiently used in the treatment of lipid disorders, especially hypercholesterolaemia.[1] They target HMG-CoA reductase, an enzyme that catalyses the conversion of HMG-CoA into mevalonate, which is the rate-limiting step in cholesterol biosynthesis.[2] Statins are mostly known for improving cardiovascular outcomes in the general population as well as in patients with cardiovascular disease (CVD). For this reason they are the most often prescribed cholesterol-lowering agents worldwide.[3]

Beyond their well established beneficial effects in CVD, statin role in other pathological entities has also been investigated. In this regard, they might affect the natural history of diseases such as Parkinson's disease, dementia, chronic kidney disease, rheumatoid arthritis and osteoporosis.[4–9] Most importantly, accumulating preclinical and clinical evidences suggest that statins might play a pivotal role in tumourigenesis.[10,11] In this regard, it has been postulated that statins may have the ability to inhibit tumour cell

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proliferation and induce apoptosis.[12] They also affect angiogenesis and possess anti-inflammatory and anti-invasive properties, affecting tumour cell viability and metastatic potential.[13,14]

Prostate cancer (PCa) is the most commonly diagnosed malignancy and one of the leading causes of cancer lethality in the Western World.[15] With respect to the role of statins in prostate carcinogenesis, experimental data as well as clinical studies report conflicting results, with some of them showing beneficial[16] and others insignificant[17] or even harmful effects.[18] In this review, we present available preclinical and clinical evidences on the effects of statins on prostate carcinogenesis and place them into perspective with regard to current recommendations on statin prescription in patients with this type of malignancy.

2. Molecular effects of statins on prostate carcinogenesis

Several lines of evidence suggest that statins affect prostate carcinogenesis in two ways. First, by lowering the cholesterol level and second, by their pleiotropic cholesterol-independent effects. Most of the pleiotropic effects are mediated via inhibition of synthesis of important isoprenoids, such as farnesylpyrophosphate (FPP; C15) and geranylgeranylpyrophosphate (GGPP; C20), which are precursors of cholesterol biosynthesis. An overview of the potential (based on preclinical data) protective and harmful effects of statins on PCa is shown in Fig. 1.

2.1. *Protective effects*

2.1.1. *Cholesterol-mediated effects*

2.1.1.1. Lipid rafts/PI3K-Akt pathway activation. Cholesterol contributes to cell survival in several ways, one of the most important being regulation of signalling through membrane molecules. In plasma membrane cholesterol accumulates in specific structures known as 'lipid rafts' or detergent-resistant microdomains (DRMs).[19] They represent low-density, laterally segregated components of the plasma membrane which are characterised by light buoyant density and high levels of cholesterol and fatty acids with long saturated acyl chains (e.g. glycosphingolipids). Lipid rafts, regulate cell signalling as well as various cellular functions, including survival. Cholesterol serves as a spacer between the hydrocarbon chains of sphingolipids, thus playing a critical role in raft formation and integrity. Cholesterol depletion could, therefore, lead to raft disruption rendering these domains non-functional and causing dysregulation of raft-dependent signalling events.[19,20]

At least two morphologically distinguishable varieties of rafts have been described: morphologically flat rafts (G-domains) and caveolae. 'Caveolae' ('little caves') are involved in transport of molecules to and from the cellular membrane as well as in regulating a variety of signalling pathways implicated in cell proliferation and apoptosis. They are characterised by the presence of one or more members of the caveolin family which have the ability to transport and bind cholesterol to the cellular membrane.[21]

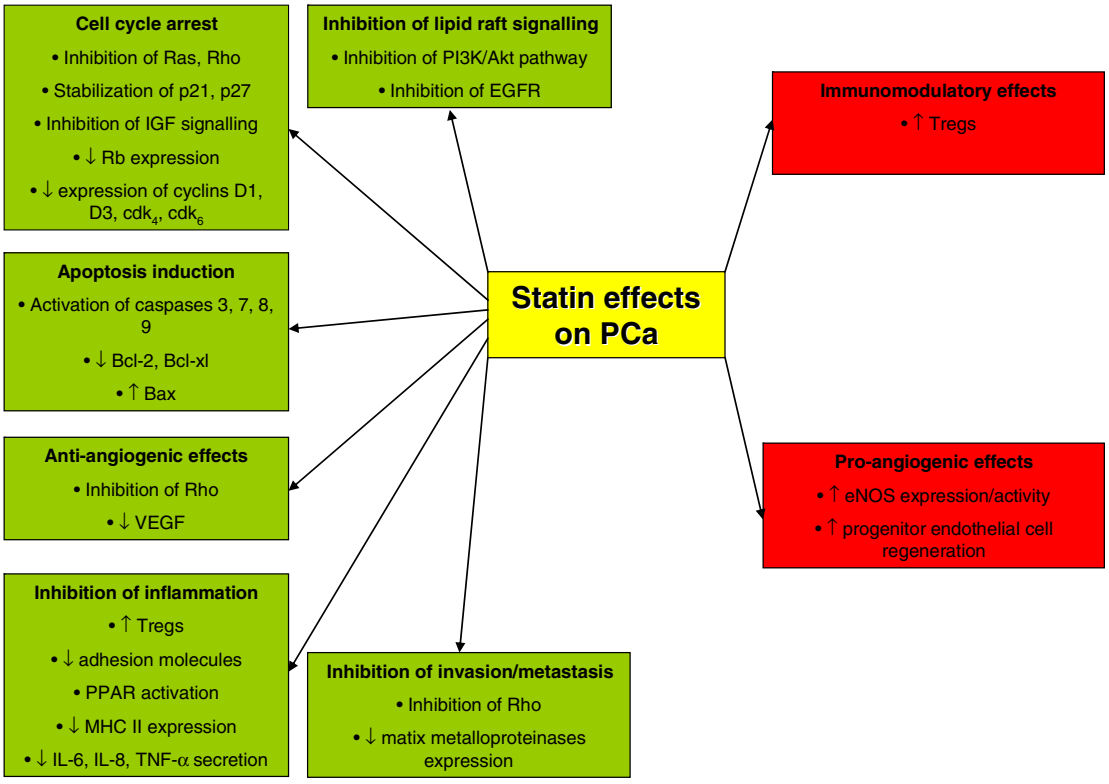

Fig. 1 – Potentially protective (left green) and harmful (right red) effects of statins on prostate tumourigenesis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Caveolin-1 expression has been associated to PCa aggressiveness (Gleason score) and metastatic dissemination.[22,23] In an experimental study, Williams et el, using a TRAMP transgenic PCa mouse model, found that the loss of caveolin-1 impeded progression to invasive and metastatic disease.[24] In the same model, Di Vizio et al. showed that loss of caveolin-1 expression resulted in loss of cancer aggressiveness through fatty acid synthase (FAS) inactivation.[25] Moreover, Swinnen et al. reported increased FAS staining from low to high grade PIN and to invasive carcinoma in frozen needle prostatic biopsies. Of note, normal or benign hyperplastic tissues were all FAS-negative.[26]

Fatty acid synthase (FAS) is the key enzyme of *de novo* fatty acid synthesis and has been reported to be overexpressed in several human malignancies including PCa. As reported above, FAS overexpression seems to be an early event in PCa[26] and is probably due to a generalised activation of genetically-regulated lipogenesis among cancerous cells.[27] Lipids provide the pool of available substrates for the biogenesis of cellular membranes, which is a prerequisite for the proliferative activity of tumour cells. It has been shown that FAS expression mainly affects the phospholipid content of detergent-resistant membrane fractions, thus possibly altering microdomain-controlled cell functions, including signal transduction and intracellular trafficking.[28] Although the exact mechanism by which FAS activation promotes prostate carcinogenesis remains unknown,[29] the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway seems to play a central role.[30] PI3K-Akt pathway is a critical regulator of cell survival and is overexpressed in many types of malignancies, as well as in LNCaP cells.[31]

Lipid rafts have also been reported to contain a subpopulation of androgen receptor (AR) that regulates intracellular signalling pathways. This process may be caveolin-mediated and transmits androgen-dependent signals to the PI3K/Akt pathway.[32] However, signalling from AR to Akt within lipid rafts can also occur in a PI3K-independent manner.[33]

Lipid raft cholesterol may also promote cell survival and tumourigenesis by inhibiting the phosphatases responsible for Akt inactivation. Li et al. demonstrated that, in PCa cells, caveolin maintained the activated condition of Akt by inhibiting two serine/threonine protein phosphatases, PP1 and PP2A.[34] Similarly, Zhuang et al. reported that elevated serum cholesterol levels increased Akt phosphorylation and reduced apoptosis, whereas simvastatin administration led to Akt attenuation and apoptosis induction in LNCaP cells (at a concentration of 20 μM).[35]

Rafts have also been shown to activate epidermal growth factor receptor (EGFR)-mediated signalling. EGFR contains high concentrations of cholesterol-rich plasma membrane microdomains.[36] In this context, cholesterol is considered to play a key role in several downstream targets of EGFR. It has been reported that cholesterol depletion after administration of 2-hydroxypropyl-β-cyclodextrin (HPCD) in LNCaP cells led to inhibition of both EGFR/Akt and EGFR/ERK pathways and apoptotic cell death through down-regulation of Bcl-xl and up-regulation of caspase-3.[37] Moreover, the co-administration of HPCD and genistein, a well-known isoflavone, in PCa cells, synergistically inhibited Akt phosphorylation, via an EGFR-mediated mechanism.[38] Finally, reconstitution of rafts with cholesterol has been shown to restore the EGFR/Akt axis signalling and cytoprotection in LNCaP cells.[39]

In conclusion, membrane cholesterol seems to activate the pro-survival PI3K/Akt and EGFR signalling pathways in prostate cancer cells and thus, reduction of cholesterol level may suppress tumour cell growth.

2.1.2. *Non-cholesterol-mediated 'pleiotropic' effects*

As previously stated most of statin pleiotropic effects are mediated via the inhibition of synthesis of isoprenoids, with FFP and GGPP representing the most important ones. The isoprenoids are lipid moieties that regulate the posttranslational modification of a variety of proteins including the small GTPase family of proteins with Rho and Ras being the most important ones.[40] To achieve activation, these proteins must be attached to the isoprenoids. This posttranslational modification of small GTP and other proteins is referred to as isoprenylation or prenylation. Isoprenylation is an essential process for the translocation and anchorage of the proteins to the cell membrane where they exert their functions.[41]

Isoprenylated proteins play a pivotal role in cellular processes, such as proliferation, differentiation, apoptosis and migration and control crucial cellular functions, such as membrane trafficking, cytoskeletal organisation and gene expression. Of note, K-Ras isoform mutations have been detected in approximately 30% of human malignancies, though in lower rates in PCa.[42]

2.1.2.1. Cell cycle and apoptosis. Isoprenylated Rho and Ras proteins are confined in the inner face of the cellular membrane and control the signal transduction of a variety of membrane receptors with tyrosine kinase activity.[43] As a result of this activation, a downstream cascade leads to the recruitment of several proteins that promote cell survival.

Rho-associated protein kinase (ROCK) seems to be the best characterised downstream effector of Rho, regulating several processes, such as cellular proliferation, inflammation and oxidative stress.[44] Regarding Ras signalling, its most well described downstream mediator is the serine/threonine kinase Raf-1. Raf-1 phosphorylates two mitogen-activated protein kinase (MAPK) kinases (MEK-1, MEK-2) which, subsequently, phosphorylate MAPKs, [also known as Erks (extracellular signal-regulated kinases)]. Erks migrate to the nucleus and regulate cell proliferation through activating several factors. In this regard, the so called Raf/MEK/Erk pathway causes upregulation of cyclin D1, which is actively involved in the cell cycle.[45] Cyclin D1 upregulation may also by induced by members of the Rho GTP-ase family (RhoA, Rac1, Cdc42).[46] D- and E-type cyclins bind to cyclin-dependent kinases 4/6 (Cdk4/Cdk6) and Cdk2, respectively, resulting in hyperphosphorylation and subsequent inactivation of the tumour suppressor retinoblastoma (Rb) protein. Rb protein inhibits cell replication by triggering histone deacytylase release and inactivating the transcription factor E_2F.[47] The above events take place once the cell enters G1 phase. RhoA and Ras can also regulate the cell cycle by down-regulating the cdk inhibitors $p21^{Cip1}$ and $p27^{Kip1}$. Further into G1 phase, isoprenoids also activate the prosurvival PI3K/Akt pathway.[48] PI3K/Akt may suppress BAD, increase the activity of Bcl-2 and Bcl-xl,[49] and inhibit the activation of caspase-9,[50] thus preventing cell apoptosis.

From the above it seems plausible that by blocking isoprenylation statins might inhibit prostate tumour cell growth. Indeed, Lee et al. found that lovastatin (at a concentration of 0.3 μM) induced senescence and G1 cell cycle arrest by modulating RhoA activity in PCa cells.[51] Similarly, RhoA inactivation by lovastatin in TRAMP cells caused cell cycle arrest and apoptosis whereas addition of the geranylgeranylation stimulator geranylgeraniol (GGOL) in the presence of 10 μM lovastatin, prevented the statin effects.[52] Additionally, atorvastatin induced autophagy and autophagy-associated cell death in PC-3 cells in a dose-dependent manner (maximal effect at 5 μM) most likely through inhibition of geranylgeranylation.[53]

Hoque et al. showed that both lovastatin and simvastatin at 2 μM induced apoptosis and G1 growth arrest in PC3, DU145 and LNCaP PCa cells. Both statins activated caspases -3, -8 and -9, induced p21 and p27 expression and suppressed expression of Rb, phosphorylated Rb, cyclin D1, cyclin D3, CDK4 and CDK6.[54] Similarly, caspase-7 underwent proteolytic activation after lovastatin treatment in LNCaP[55] as well as in PC-3 cells,[56] at concentrations of 30 and 2 μM, respectively. Lee et al. reported that 10 μM lovastatin may circumvent the loss of wild-type p53 function in PC-3 cells and induce p21 transcription,[57] whereas Sivaprasad et al. showed that statin-mediated cell cycle arrest was p27-independent and differed among various HMG-CoA reductase inhibitors, ranging from clinically feasible (0.5 μM) concentrations for lovastatin to extremely high (200 μM) for pravastatin.[58] Similarly, Park et al. by using lovastatin at 10 μM reported that E2F-1 (but not p21 or p27) is the possible target of statins in PCa cells[59] whereas Ukomadu and Dutta showed that 10 μM mevastatin inhibited the proliferation of PC-3 cells through cdk2 inhibition but again not p21 or p27.[60] However, 10 μM lovastatin induced cell cycle arrest in G1 phase with simultaneous $p21^{Cip1}$ and $p27^{Kip1}$ up-regulation, in PCa cells.[61] Finally, statins can also inhibit cell growth by affecting insulin-like growth factor 1 receptor (IGF-1R). IGF-1R signalling has been reported to regulate cell differentiation, malignant transformation and cell–cell adhesion.[62] Its effect on cell proliferation and apoptosis inhibition seems to be exerted through both MAPK and PI3K/Akt pathways and simvastatin has been shown to suppress this signalling in PC-3 cells.[63] In support of this, high IGF-1 circulating levels have been associated with increased risk of prostate cancer.[64]

2.1.2.2. Anti-angiogenic effects. Statins have been shown to exert both pro-angiogenic and anti-angiogenic activities (the so called biphasic effect), depending on their dose and tumour cell types. With respect to dose, low doses seem to promote angiogenesis whereas high doses exert the opposite effect.[65] It has been shown that statins' properties on angiogenesis are principally mediated via regulating endothelial nitric oxide (eNO) production, a critical mediator of angiogenesis.[66]

As previously noted ROCK is the best characterised downstream mediator of Rho signalling.[44] ROCK activation results in inactivation of myosin light chain phosphatase and reduces the expression of eNO synthase (eNOS).[67] Moreover, changes in actin polymerisation induced by Rho can affect eNOS mRNA stability and reduce NO production in a ROCK-independent manner.[68] Similarly, activation of Rho by *Escherichia coli* cytotoxic necrotizing factor-1 decreases eNOS expression.[69] In this regard, inhibition of Rho activation by statins may inhibit angiogenesis by downregulating eNOS. Finally, statins have been reported to downregulate vascular endothelial growth factor (VEGF) as well as other angiogenic factors in endothelial cells.[13]

Regarding the angiogenic effects of statins on PCa, data are limited. Of note, Wang et al. showed that rosuvastatin inhibited angiogenesis by inducing G1 phase arrest and promoting apoptosis of human umbilical endothelial cells (HUVECs) in a PCa xenograft mouse model.[70] Conclusively, high doses of statins with or without the concomitant administration of agents that block their pro-angiogenic effects could be part of future treatments for PCa.

2.1.2.3. Inflammation. Evidence from epidemiologic and laboratory studies are suggestive of a causative role of inflammation in PCa progression.[71] In this regard, a recent study showed that preoperative statin use in men undergoing radical prostatectomy significantly reduced the risk for intratumoural inflammation, suggesting a possible mechanism for statins anti-cancer properties.[72] But how do statins affect inflammation?

Statins have been reported to regulate T-cell phenotype. In this regard, they increase the number of CD4+CD25+ regulatory T cells (Tregs) *in vivo* by inducing the transcription factor, forkhead box P3.[73] Tregs, by suppressing immune responses, have been shown to prevent various immunoinflammatory[74] as well as experimental autoimmune diseases.[75] Moreover, statin use has been associated with decreased levels of many adhesion molecules such as CD 11b adhesion molecules,[76] leucocyte function antigen-1,[77] ICAM-1 and VCAM-1.[78] They also reduce C-reactive protein (CRP) levels in a cholesterol-lowering independent manner.[79] CPR enhances the potential for inflammation by inducing complement activation and decreasing eNOS expression.[80] By inhibiting small GTPases, statins can also repress the inducible major histocompatibility complex class (MHC) II expression in antigen presenting cells, thereby inhibiting T-lymphocyte activation.[81] Moreover, they reduce the secretion of pro-inflammatory factors, such as interleukin IL-6, IL-8, IL-1β, TNF-α and MCP-1.[82] Statins can also inhibit inflammation by activating peroxisome proliferator-activated receptors (PPARs). PPARs are a family of nuclear receptors that regulate a variety of cellular functions, such as differentiation, survival and replication.[83,84]

Evidence for the beneficial role of inflammation reduction in PCa growth emerges from experimental studies in which cyclooxyganase inhibitors or PPAR agonists were used in addition to a statin. Intriguingly, Zheng et al., noted a more potent growth inhibitory effect with the combination of atorvastatin with celecoxib than either agent alone in LNCaP tumours.[85] Similarly, Murtola et al. reported that the combination of simvastatin with acetylsalicylic acid, at clinically relevant doses, led to growth inhibition in malignant prostatic epithelial cells.[86] Finally, lovastatin and the PPARγ agonist troglitazone, synergistically inhibited PCa cell growth.[87]

2.1.2.4. Invasion and metastasis. Several lines of evidence suggest that statins may impair the invasive and metastatic potential of tumours by inhibiting extracellular matrix

degradation, cell migration and invasion.[88] Specifically, in cancer cell lines other than prostate, isoprenylated Rho has been shown to play a crucial role in cytoskeleton organisation, cell adhesion and migration, as well as extracellular matrix integrity.[14,89] In this regard, statins have been shown to reduce matrix metalloproteinases and Rho-mediated E-selectin expression thus inhibiting crucial components of the invasive and metastatic processes.[90,91] Similarly, by preventing RhoA activation they might inhibit EGF-induced invasiveness.[92] Although there is a dearth of data on the role of statins in PCa invasion and metastasis it seems reasonable that statins might exert similar effects on this tumour type as in other cancers.

2.2. *Potentially harmful effects*

2.2.1. *Immunomodulatory effects*

As discussed earlier, statins have been shown to increase Tregs *in vivo*.[73] Tregs by suppressing immune responses might also suppress tumour-specific T-cell responses, thus increasing cancer risk.[93] Of note, increased Tregs were found in the peripheral blood of PCa patients compared with normal donors. Similarly, Tregs were increased in PCa tissue compared with the normal prostate tissue from the same prostate.[94] These experimental findings are supported by clinical studies (albeit few), that report an increased PCa risk among statin users.[95]

2.2.2. *Pro-angiogenic effects*

Statins can promote angiogenesis depending on dose and tumour cell type and thus might enhance tumour growth. Of note, statins can increase eNOS expression and activity via ROCK-dependent and ROCK-independent mechanisms. In endothelial cells, statins have been shown to rapidly activate Akt, which in turn, phosphorylates and activates eNOS.[96] Moreover, statins increase eNOS production by reducing caveolin-1 and this effect is cholesterol-mediated. Caveolin inhibits eNOS by blocking its access to cofactors, thereby directly inhibiting NO production.[97] Finally, statin therapy might reverse the impaired functional regeneration capacities of progenitor endothelial cells thus promoting angiogenesis.[98]

3. Statins and prostate cancer. Clinical data

Most data on the potential relation between statins and PCa emerge from observational (case control or cohort) studies. In many of these studies researchers went through large cancer-registry databases to identify the number of patients among statin users that developed PCa after many years of follow up. The key factor in interpreting those studies results is whether there was an adjustment for prostate specific antigen (PSA) testing and PSA levels or not. Indeed, although systematic screening for PSA is not recommended in most countries due to its unproven efficacy in affecting PCa outcomes,[99] opportunistic PSA testing is common and it might affect the

Table 1 – Observational studies evaluating the association of statins with PCa risk.

Study	Study type	Patients (*n*)	PCa patients (*n*)	Observational period (years)	Statin impact on PCa risk (95% CI)	Comment
Not adjusted for PSA testing						
Coogan[102]	Case-control	8.813	1.226	15	OR: 1.2 (0.9–1.7)	No impact on overall cancer risk
Graaf[17]	Case-control	20.105	186	14	OR: 0.37 (0.11–1.25)	Significant↓ in overall cancer risk
Blais[103]	Case-control	5.962	78	7	OR: 0.74 (0.36–1.51)	Significant↓ in overall cancer risk
Friis[104]	Cohort	13.508	1.407	14	RR: 0.87 (0.61–1.23)	Significant↓ in overall cancer risk
Kaye[105]	Case-control	1.322	266	13	**RR: 1.3 (1.0–1.9)**	No impact on overall cancer risk
Haukka[18]	Case-control	944.962	1.051	10	**RR: 1.12 (1.08–1.17)**	No impact on overall cancer risk
Murtola[106]	Case-control	49.446	24.723	8	**OR: 1.07 (1.00–1.16)**	**OR: 0.61 in advanced PCa (CI: 0.37–0.98)**
Adjusted for PSA testing						
Friedman[111]	Cohort	361.859	1.706	9	HR: 1.04 (0.93–1.17)	No impact on overall cancer risk
Farwell[110]	Cohort	62.842	1.001	7	**HR: 0.90 (0.81–0.99)**	Significant↓ in overall cancer risk
Flick[112]	Cohort	69.047	888	14	**RR: 0.72 (>5 years) (0.53–0.99)**	Significant only for patients on NSAIDs
Breau[113]	Cohort	2.447	224	18	**HR: 0.36 (0.25–0.53)**	–
Murtola[16]	Cohort	23.320	1.594	9	**HR: 0.75 (0.63–0.89)**	Strongest relation in early-stage tumours
Shannon[107]	Case-control	302	100	7	**OR: 0.38 (0.21–0.69)**	**OR: 0.24 in Gleason ⩾ 7 (CI: 0.11–0.53)**
Platz[108]	Cohort	34.989	2.579	13	RR: 0.96 (0.85–1.09)	**RR: 0.51 in advanced PCa (CI: 0.30–0.86)**
Jacobs[109]	Cohort	55.454	3.413	6	RR: 1.06 (0.93–1.20)	**RR: 0.60 in advanced PCa (CI: 0.36–1.00)**
Boudreau[114]	Cohort	83.372	2.532	16	HR: 0.88 (0.76–1.02)	**HR: 0.79 for lipophobic statins (CI: 0.66–0.94)**
Agalliu[115]	Cohort	1.943	1.001	4	OR: 0.98 (0.80–1.21)	**OR: 1.5 in BMI ⩾ 30 (CI: 1.00–2.2)**

Abbreviations: n, number; PCa, prostate cancer; CI, confidence intervals; OR, odds ratio; RR, relative risk; HR, hazard ratio; NSAID, non-steroidal anti-inflammatory drugs; BMI, body mass index.
Statistically significant statin/PCa risk associations are marked in bold.

apparent PCa incidence. In this regard, patients who had been prescribed a statin were more likely to be more adherent to medical treatments and supervision and thus more likely to have had a more frequent PSA testing and a PCa diagnosis.[100]

Conversely, statins have been reported to reduce the PSA level thus delaying the decision for a prostate biopsy and PCa diagnosis. In this regard, patients on these agents might be underdiagnosed with PCa.[101] Therefore, adjusting for PSA testing and PSA levels, could probably eliminate PCa detection bias introduced by opportunistic PSA testing as well as by the effects of statins on PSA levels and thus, seems to be crucial for the credibility of the observational studies results.

3.1. *Observational studies*

3.1.1. *Studies not adjusted for PSA testing*

Large observational studies not adjusted for PSA testing not only showed that statins do not reduce overall PCa risk,[17,102–104] but in some of them this risk was even increased[18,105,106] (Table 1). However, with respect to the overall cancer risk, many of them reported a protective effect. Coogan et al. in a hospital-based case-control study including 8813 patients followed for 15 years, demonstrated a statistically non-significant effect of statins on overall and PCa risk.[102] Similarly, Graaf et al., in a large cohort of 20,105 patients followed for 14 years reported a non-significant PCa reduction risk in statin users, but a significant reduce in overall cancer risk.[17] Consistent with these findings, Blais et al., in a population of 5962 patients followed for 7 years, reported a statistically significant 28% reduction in overall cancer risk for statin users compared with bile–acid-binding resin (cholesterol-lowering agents) users but a non-significant 26% reduction in PCa risk.[103] Finally, a Danish study comparing statin users with users of other lipid-lowering medications in a cohort of 13,508 patients reported similar results.[104]

As previously denoted, some of the observational studies not controlling for PSA testing reported a positive association between statins and PCa. Kaye et al. gleaned cancer cases from a General Practitioner-derived database and examined 1322 statin users during a period of 13 years.[105] In this study, although no impact of statins on overall cancer risk was detected, the risk for PCa was found marginally increased in the statin group (RR:1.5; CI: 1.0–1.9). Similarly, in a Finnish study, including 944,962 patients of which 1051 developed PCa during a follow up period of 10 years, a significant 12% increase in PCa incidence for statin users was detected.[18] Finally, Murtola et al., in a large population-based case-control study, including 24,723 PCa patients out of a total of 49,446 patients followed for 8 years), found that statin use was associated with a 7% increase in PCa incidence.[106] Intriguingly, the risk of advanced PCa was significantly lower among statin users.

3.1.2. *Studies adjusted for PSA testing*

In contrast to the results of studies not adjusted for PSA testing, many studies controlling for PSA testing reported a significant reduction in overall PCa risk for statin users,[16,107,110,112,113] whereas some also indicated a lower risk for advanced PCa in the statin group[107–109] (Table 1).

The first report that underscored a significant inverse statin-PCa risk association was a small case-control study by Shannon et al. that revealed a 62% and 76% reduction in overall and high grades (Gleason score $\geqslant 7$) PCa, respectively.[107] Moreover, Platz et al., in a cohort of approximately 35,000 health professionals detected an inverse association between advanced and fatal PCa and statin uses.[108] However, the overall PCa risk was not decreased. Similarly, Jacobs et al. reported a marginally significant 40% reduction in advanced PCa incidence in statin users, although the overall PCa incidence was not reduced.[109] In a large veterans population of over 60,000 individuals, Farwell et al. reported a significant 26% and 10% decrease in the overall and prostate Ca incidence, respectively, after a follow-up of approximately 8 years.[110] However, a Canadian study in 361,859 patients who used lovastatin, simvastatin or both failed to detect a significant effect of these statins on overall or PCa risk.[111]

Flick et al., in 69,047 participants from the California Men's Health Study concluded that long-term (>5 years) statin use was accompanied by a significant 28% reduction in PCa risk.[112] However, this association seemed to be restricted to those who regularly took NSAIDs. Similarly, Breau et al., in a population based cohort of 2447 patients followed for 2 years, detected a significant reduction in PCa incidence among statin users.[113]

Boudreau et al., indicated a significant reduction of PCa risk in the subpopulation of patients that consumed hydrophobic (lovastatin, simvastatin) statins,[114] whereas Agalliu et al. showed a higher PCa incidence in obese statin users (body mass index $\geqslant 30\ Kg/m^2$).[115] Of note, a positive relation between obesity and high-grade PCa has previously been reported,[116] but it remains unclear whether statins exert any specific effects on this subpopulation.

Very recently, Murtola et al., tried to eliminate PCa detection bias introduced by opportunistic PSA testing and statin-induced fluctuations in PSA level by enrolling patients from the Finnish PCa Screening Trial.[16] From 23,320 patients that underwent systematic PCa screening, 1594 developed PCa during 9 years of follow-up. The overall PCa incidence was significantly decreased among statin users. Intriguingly, this inverse association was dose-dependent and strongest for early-stage disease. Thus far, this is the only study to assess the association between statins users and PCa incidence in a population systematically screened for PCa, thus eliminating PCa detection bias between statin users and non-users.

Although observational registry-based studies generally have the advantages of large sample size and minimal information bias (due to differential information on exposure or disease between comparison groups), several drawbacks put the validity of their results into question. First, in many of the aforementioned studies the number of PCa cases was small. Second, information on crucial data for the analysis and interpretation of the results might have been lacking. For example, studies not controlled for PSA testing did not detect a significant reduction of PCa risk, or they even reported an increased risk, whereas the vast majority of studies adjusted for PSA testing reported an inverse association between statins use and overall PCa incidence or advanced PCa risk. Moreover, patients in the statin arm obviously more frequently consumed NSAIDs or troglitazone for the prevention of CVD. As discussed earlier, preclinical evidence suggests that NSAIDs and PPARγ agonists might synergistically

act with statins to prevent PCa development.[85–87] In accordance with this, Flick et al., concluded that long-term statin use was accompanied by a significant reduction in PCa risk in patients who regularly took NSAIDs.[112] Therefore, an adjustment of PCa observational studies for NSAIDs or PPARγ use is essential for higher quality results.

3.2. Meta-analyses

Thus far, data from randomised controlled trials (RCTs) evaluating the association between statins and PCa emerge from major CVD prevention statin trials. The primary end-point of these trials was to evaluate the effect of statin treatment on the primary or secondary prevention of CVD. However, some of these trials evaluated overall cancer or PCa risk as secondary end-points in order to assess these drugs' safety. Meta-analyses of these RCTs as well as meta-analyses of the previously described observational studies have been published. Of note, none of these meta-analyses detected a significant effect of statins on PCa risk.

Baigent et al. pooled data from 14 RCTs including 90,056 patients and 1535 genitourinary tumours.[117] The mean follow up period was 5-years and no association between statins and prostate cancer incidence was reported. These results are in accordance to those of Dale's et al. meta-analysis of 26 RCTs.[118] A total of 86,936 participants, were followed for 4 years and no relation between statins and overall as well as PCa risk was detected.

Browning et al. performed two meta-analyses on 26 RCTs and 12 observational studies.[119] The follow-up periods were 3.6 and 6.2 years, respectively and no influence of statins on prostate and overall cancer risks was detected. Similarly, Kuoppala et al., using a database of 42 trials, 17 of which were randomised, did not report a significant effect of statins on overall and prostate cancer risk, though protective effects were detected in other cancer types.[120] Taylor et al., by pooling data from twenty case-control studies reported a significant 29% (95% CI: 0.56–0.89) reduction in overall cancer risk, though not in PCa.[121] Flaws of this meta-analysis comprise the lack of randomised trials and the significant heterogeneity of the included studies.

In the only existing meta-analysis investigating the effect of statins solely on PCa, Bonovas et al. by pooling data from 6 RCTs and 13 observational studies, concluded that statins do not significantly influence PCa risk.[122] Sub-analyses on RCTs (40,178 patients, including 1058 PCa cases with an average follow-up of 7.4 years) and observational studies (840,000 patients, including 61,314 PCa cases) yielded the same result. Long-term statin use (>5 years) was not associated with changes in PCa risk. Intriguingly, statin use was associated with reduced incidence of advanced PCa (RR: 0.77, CI: 0.64–0.93).

With respect to meta-analyses of major statin RCTs several flaws might confound their results. First, the duration of statin administration was short and thus it might not suffice for any clinically significant effect on PCa prevention to be demonstrated. Second, the population involved in these trials was selected by strict criteria and, therefore, their results cannot be extrapolated to the general population. Moreover, as the primary end-point of these trials was to evaluate the effect of statins on CVD prevention, they seem to be underpowered to detect any significant effect on PCa risk. Finally, these meta-analyses did not evaluate the possible relation between statin use and the different PCa stages or grades. For these reasons, the results of the aforementioned meta-analyses should not be considered confirmatory of an insignificant role of statins in prostate carcinogenesis.

3.3. Studies on post-treatment PSA recurrence

Recently published studies reporting on statin effects on the biochemical recurrence risk (in terms of PSA recurrence) after PCa radical treatment (radical prostatectomy or radiation treatment) have provided additional data on the role of statins in PCa risk.

Regarding patients treated with radical prostatectomy, Krane et al. in a retrospective evaluation of 3828 patients did not detect a significant reduction in the biochemical recurrence risk among statin users.[123] Similarly, a Korean study showed that preoperative statin use was not associated with reduced biochemical recurrence[124] whereas Hamilton et al. in a cohort of 1319 men found a dose-dependent reduction in the risk of biochemical recurrence in statin users.[125]

With respect to patients managed with radiation therapy, Soto et al. did not find any association between statin use and PSA recurrence after external beam radiotherapy for localised PCa.[126] However, these results oppose those of Gutt et al. reporting on significantly decreased biochemical recurrence rates in patients treated with a statin[127], whereas Kollmeier et al. detected a congener effect of statins only in high-risk individuals.[128]

However, as statins might reduce the PSA level thus preventing the timely diagnosis of patients with biochemical recurrence, the results of the aforementioned studies should be interpreted with caution.

4. Conclusions and recommendations

Currently, the official indications for statin prescription are hypercholesterolaemia or the prevention of CVD in patients with clinically evident coronary heart disease or without clinically evident coronary heart disease, but with multiple risk factors for coronary heart disease. Moreover, prevention of CVD in patients with chronic kidney disease stage 1–IV seems to emerge as a novel indication.[6]

Hypercholesterolaemia has been suggested to promote prostate tumourigenesis in mice,[35] whereas several lines of preclinical evidence suggest a preventive role of statins in PCa through both cholesterol-lowering dependent and independent effects.[19,101] From the experimental data earlier presented it seems that statins exert their so called 'pleiotropic' effects *in vitro* at relatively high concentrations (0.5–20 μM)[10,35,54] compared to clinically achievable serum concentrations in patients treated for primary or secondary prevention of CVD (less than 0.2 μM).[129] Moreover, low lovastatin concentration has been shown to inhibit cell cycle *in vitro* mostly through cholesterol-lowering whereas high concentrations (50 μM) are needed for lovastatin's pleiotropic effects.[130] Thus, it seems that cholesterol-lowering is a significant mechanism

by which statins might inhibit carcinogenesis *in vivo*. However, there is no single study showing a significant inverse association between other (than statins) cholesterol-lowering agents and PCa risk, implying that statins pleiotropy is at least partly responsible for their antitumour properties. Finally, whether high statin concentration is required *in vivo* for any growth inhibitory effect remains an enigma. Moreover, it is possible that the sustained effect of low statin concentrations after chronic administration might result in an equivalent outcome to their *in vitro* documented short term high dose effects.

Summarizing, statins might inhibit prostate carcinogenesis by inducing apoptosis of prostate cells or inhibiting prostate cell growth, inhibiting angiogenesis and invasion/metastasis or alleviating inflammation. Most of these pleiotropic effects are mediated by the inhibition of the synthesis of isoprenoids. Moreover, statins can also inhibit prostate cell proliferation through the reduction of membrane cholesterol concentration, which results in the derangement of lipid raft formation and integrity. With respect to statin physical properties clinical evidence suggests that neither solubility nor potency influence their growth inhibitory effects.[21,131]

Clinical evidence on statin protective effects against PCa is still weak. Indeed, thus far clinical data emerge from observational studies and meta-analyses of major statin RCTs. Regarding the former, several confounding factors, such as lack of adjustment for PSA testing or for the concomitant use of NSAIDs or PPARγ agonists, along with the small number of PCa cases in many studies impair the quality of the results, whereas the latter are flawed by the short duration of statin use, low power and strict population selection criteria that render the generalisation of their results implausible. In addition, patients receiving statins are also advised to implement lifestyle changes pointing towards cardiovascular disease (CVD) prevention, such as exercise, reducing animal-lipid food and consuming low-calorie products such as fish, soy and vegetables. This lifestyle might also relate to reduced PCa risk and reduced progression of well-differentiated malignancies, thus introducing another potential bias in the aforementioned studies.[132] Intriguingly, published data support that lifestyle modifications do not seem to impact PCa if the cholesterol profile remains unchanged.[133]

In conclusion, given the absence of prospective RCTs that directly address statin effect on prostate carcinogenesis as well as the limitations of existing observational studies, secondary analyses of RCTs and meta-analyses, statins should not be recommended for the prevention of PCa development or progression. However, men getting a statin prescription for the usual statin indications could be informed that these drugs might also reduce the risk for PCa development. Physicians should also be aware of the potential harmful effects of statins on prostate carcinogenesis suggested by experimental studies and may emerge when results from better designed observational studies (in which PCa detection bias has been eliminated) or RCTs come out. However, the design of an RCT that evaluates statin influence on PCa risk as a primary end-point should overcome several difficulties. In this regard, a very large number of patients should be recruited and a long follow-up time would be needed for the study to be powered enough to detect potential antitumour effects. Moreover, as patients at risk for developing PCa are of similar age with those at high risk for CVD, it is possible that patients in the control arm might at some point have to commence a statin for the prevention of CVD.

Such RCTs could probably be designed on patients with localised, well-differentiated carcinomas where active surveillance is considered by some authorities as the treatment of choice. These patients could be randomised to statin or placebo and followed, ideally, for many years in order to detect differences in the disease progression between the two arms. Finally, as controlled for PSA testing observational studies have consistently demonstrated a protective role of statins in advanced PCa, future RCTs should differentially examine statin effects on different PCa subgroups (i.e. according to grade or stage).

Although, the role of statins in prostate carcinogenesis will hopefully be elucidated by future clinical trials, it should be noted that redundant preclinical evidence as well as the scanty available clinical data suggest that statins might finally be proven to prevent prostate carcinogenesis.

Conflict of interest statement

None declared.

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