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Review Article

Resveratrol used as nanotherapeutic: a promising additional therapeutic tool against hormone-sensitive, hormone-insensitive and resistant prostate cancer

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Abstract: Prostate cancer is one of the most common cancers in men. Despite the development of diverse therapeutic agents for different types and stages, the progression or spread of the disease is inevitable. Another problem is the development of resistance of cancer cells to available therapeutics. Therefore, additional medicaments are urgently needed. Resveratrol is a polyphenolic phytoalexin found in numerous plants and fruits like red grapes or blueberries. Resveratrol possesses antiproliferative, anti-angiogenic and anticancer activities well proven in different types of cancer including prostate cancer. To date, it is not used clinically due to poor solubility, low bioavailability, and other limiting factors. In order to overcome these limitations, novel nanoparticle-based formulations were developed over the past years. In this review article, studies about the effect of resveratrol on prostate cancer cells are discussed focusing especially on those studies using nanotechnology. An electronic literature research was performed utilizing PubMed in August 2022. Scientific publications, which examine resveratrol using nanotechnology, are discussed. The studies clearly indicate that resveratrol-loaded nanoparticles exhibited a remarkable anti-cancer activity in various hormone-sensitive and hormone-insensitive prostate cancer cell lines including docetaxel-resistant prostate-cancer cells. The types of nanoparticles that were used varied and influenced the outcome. Additionally, the meaning of the surface functionality of the nanoparticles is emphasized. No reduction of the anti-proliferative activity of resveratrol was shown when used encapsulated. Additionally, synergistic effects of resveratrol and docetaxel were proven. Resveratrol-loaded nanoparticles, especially when combined, may represent the next generation of anticancer substances. However, further in vivo/clinical studies are necessary to confirm their clinical effectiveness.

Keywords: Resveratrol, hormone-sensitive and hormone-insensitive and refractory prostate cancer, nanotechnology, nanoparticle

Introduction

Prostate cancer is one of the most frequent cancers in men. In metastatic hormone-sensitive prostate cancer (mHSPC), the traditionally used monotherapy with androgens, the androgen deprivation therapy (ADT), was changed to a multidrug approach. The following drugs are also used for the treatment of prostate cancer: Taxanes, for example docetaxel and cabazitaxel [1], abiraterone acetate [2] blocking the enzyme cytochrome P450 17 alpha-hydroxylase (CYP17) as well as the androgen receptor pathway inhibitors enzalutamide [3], darolutamide and apalutamid [4]. Prostate cancer is a very heterogenous disease. Therefore, different therapeutical strategies are necessary. An additionally used pharmacological class are the PARP inhibitors [PARP: Poly (ADP-ribose) polymerase-inhibitors], for example olaparid [5]. The enzyme Poly (ADP-ribose) polymerase (PARP) is important in DNA repair, which includes the repair of DNA damage inside cancer cells after chemotherapy. Targeting and inhibiting this enzyme could increase the sensitivity to chemotherapy. Olaparib delays the cancer progression, but only for several months. De Bono et al. [5] showed in patients with metastatic castration-resistant prostate cancer suffering from disease progression during treatment with enzalutamide or abiraterone and showing alterations in genes with a role in

homologous recombination repair that the therapy with olaparib led to a longer progression-free survival and better measures of response and patient-reported end points than either enzalutamide or abiraterone. In the OlympiAD study it was shown that adverse events during treatment with olaparib were low grade and manageable [6]. The most common adverse effects were anemia, nausea and vomiting [6].

Radium-223 [7] and other radionuclides [8] can be used additionally to treat bone metastases. Radium-223 dichloride was the first approved α-particle-emitting radiopharmaceutical used in patients with bone metastases in castration-resistant prostate cancer (CRPC) and no evidence of visceral metastases [9]. Radium-223 significantly extended patients' overall survival and reduced pain as well as symptomatic skeletal events [9]. The reduced bone marrow toxicity is also explained by its short penetration range as an α-particle-emitting agent. However, also previously used radiopharmaceuticals, such as Strontium-89-chloride, Samarium-153-EDTMP (ethylene-diamine-tetra-methylene-phosphonate) and Rhenium-186-HEDP (hydroxyethylidene-diphosphonate), did show few and mild adverse effects like bone marrow toxicity mostly reversible and the temporary increase of pain after application in a smaller percentage of patients being manageable by standard pain killers [8].

Furthermore, radioligand therapy like ^{177}Lu-PSMA-617 [10] and checkpoint inhibitors like pembrolizumab represent additional therapeutic approaches for selected cases [11]. Despite ongoing extensive and cost-intensive research over decades developing the above-described diversity of therapeutic agents for different types and stages of prostate cancer like metastatic hormone-sensitive prostate cancer (mHSPC), advanced prostate cancer and non-metastatic/metastatic castration-resistant prostate cancer (CRPC), a curative therapy for metastatic prostate cancer is still not available. Furthermore, the progression or spread of the cancer is often inevitable. Another problem is the development of resistance of prostate cancer cells to the available therapeutics, for example resistance to taxane chemotherapy [12] or enzalutamide-resistance [13]. The problems of conventional chemotherapeutic agents are the limited availability of the agents for the tumour cells, a variety of side effects as well as the well-known toxicity to healthy tissues. Therefore, additional medicaments are urgently needed and each pioneering step to develop novel drugs should be seriously considered.

Frequently, the knowledge about the mode of action of naturally occurring substances has led to the development of therapeutics using these substances in a beneficial way for the human body, for example toxins originally produced by special bacteria have been used in the field of urology for decades to treat urological diseases [14]. Not only does the animal kingdom provide useful substances for the treatment of cancer in human beings [15], but also the plant kingdom offers us a wide range of substances representing valuable therapeutical tools to treat urological cancers. One of the most famous examples is the pharmacological class of taxanes, which was derived originally from the bark of a yew tree (botanical nomenclature: Taxus) [16] and represents now one standard treatment option against prostate cancer [1]. One of the major adverse events associated with a taxane chemotherapy are dermatological adverse events [17].

In this review article, resveratrol, another substance derived from plants, is the centre of scientific attention regarding its anti-cancer activity against prostate cancer. Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenolic phytoalexin, which was first isolated from the roots of white hellebore (*Veratrum grandiflorum* O. Loes) in 1939 [18] and is also found in numerous other plants and fruits like red grapes, blueberries, cranberries, rhubarb, peanuts, mulberries as well as in red wine [19]. As a phytoalexin, resveratrol inhibits originally the development of certain infections of the plant by activating defence mechanisms of the plant against pathogens. Plant extracts containing resveratrol have been used for treatment of different diseases for more than 2000 years [20]. Resveratrol possesses not only antioxidant, anti-inflammatory, antiviral and neuroprotective effects, but also antiproliferative, anti-angiogenic and anticancer activities shown in different types of cancer [21] like ovarian cancer [22], breast cancer [23], colorectal cancer [24] as well as in prostate cancer cells [25-27].

Nevertheless, to date, it is not used clinically due to poor solubility, a rapid degradation and a

high metabolism resulting in a low bioavailability [20, 28, 29].

Resveratrol exists in a cis and trans isomeric form [30], the trans isoform is the biologically active one.

In order to overcome the above-mentioned limitations due to poor bioavailability, novel nanoparticle-based formulations were developed over the past years [31].

In this review article, studies about the effect of resveratrol on prostate cancer cells are discussed focusing especially on those studies using nanotechnology.

Material and methods

The focus of this review article is laid on the use of the natural substance resveratrol used as nano-particles to treat prostate cancer.

An electronic literature research was performed utilizing PubMed in August 2022. Scientific publications, which examine resveratrol using nanotechnology, are discussed.

Following search terms were combined: "resveratrol" and "nanoparticle" and "prostate cancer". Instead of the term "cancer" the term "carcinoma" was also used in the above-mentioned combination. Additionally, the following combinations were used: "resveratrol" and "nanotechnology" and "prostate cancer" or "resveratrol" and "nanomedicine" and "prostate cancer".

First, the identified titles and abstracts were screened. Then the full text was screened for exclusion.

The following studies were excluded: 1. Articles in which the subject was not the above described subject. 2. Articles in which the subject was a non-urological tumour or in which others than prostate cancers were examined. 3. Review articles and consensus reports.

In this review, all types of studies are included since the number of studies on the subject is small and important information about the subject would be ignored by focusing only on randomized (prospective) studies. By using the database "ClinicalTrials.gov" an additional electronical search was performed using the terms "prostate cancer" and "resveratrol".

Results

Using the terms "resveratrol" and "nanoparticle" and "prostate cancer", 12 articles (including 3 review articles) were found. Using the terms "carcinoma" instead of "cancer" did not lead to additional publications. Using the terms "resveratrol" and "nanotechnology" and "prostate cancer" 3 articles were found, using the combination "resveratrol" and "nanomedicine" and "prostate cancer" 5 articles were found.

After exclusion using the above-mentioned search criteria, the remaining articles were included in the discussion section. Furthermore, additional publications are cited for a better understanding of the scientific context.

The additional search for clinical trials using the database ClinicalTrials.gov and utilizing the terms "prostate cancer" and "resveratrol" showed no ongoing clinical trial.

Discussion

Effect of resveratrol-loaded polymeric nanoparticles on the prostate cancer cell line LNCaP

Nassir et al. [32] created polymeric nanoparticles encapsulating resveratrol (RLPLGA) and examined their cytotoxic effects on the prostate cancer cell line LNCaP as well as their mode of action. Authors used the nanoprecipitation technique to create special nanoparticles loaded with Resveratrol. As a control empty "placebo nanoparticles" without resveratrol were used. Additional experiments were performed using "free" Resveratrol without encapsulation. The average particle size amounted to 203±3 nm, the particle size distribution (polydispersity index) of the loaded nanoparticles was 0.17±0.016. Microphotographs using the transmission electron microscopy showed that the particles were nearly spherical. The entrapment efficiency of an optimized nanoparticle was 89±4%. In concentrations from 10-100 μM, the cell viability of the prostate cancer cell line was significantly stronger inhibited by RLPLGA nanoparticles in comparison to free resveratrol. The IC50 and IC90 for the loaded nanoparticles was determined to be 15.6±1.49 and 41.1±2.19 μM, respectively, whereas their values amounted to 30 μM and 77 μM, respectively, for free Resveratrol. Several additional examinations were performed to prove that

Resveratrol loaded nanoparticles induced apoptosis of LNCaP cells. A cell cycle arrest at the G1-S transition phase was shown. At a concentration of 40 μM and 50 μM the loaded nanoparticles and resveratrol showed no cells in the phases G2-M, respectively, whereas 26% and 31% of the untreated control groups were found to be in the phases G2-M, respectively. Furthermore, an externalization of phosphatidylserine and a DNA nicking in the LNCaP cells were found after treatment with the loaded nanoparticles. Also, a decrease of the mitochondrial membrane potential in a dose-dependent manner was shown after treatment with the loaded nanoparticles as well as with free resveratrol. Furthermore, the level of intracellular reactive oxygen species in the treated LNCaP cells was increased in a concentration-dependent manner after treatment with the loaded nanoparticles and free resveratrol. The caspase-3 activity was significantly higher in LNCaP cells treated with the loaded nanoparticles than in cells treated with resveratrol alone. Free resveratrol and RLPLGA nanoparticles exhibited no cytotoxic effects on murine macrophages up to a concentration of 200 μM. This study indicates that resveratrol, especially used in an encapsulated form, could be a promising therapeutic agent to treat prostate cancer.

Effects of resveratrol-loaded nanoparticles [based on poly(epsilon-caprolactone) and poly(D,L-lactic-co-glycolic acid)-poly(ethylene glycol) blend] on the three different prostate cancer cell lines LNCaP, PC-3 and DU-145

In the following described study by Sanna et al. [33] three different cell lines of prostate cancer were used to investigate the effect of special polymeric nanoparticles encapsulating resveratrol. The LNCaP cell line represents a hormone-sensitive cell line. Originally, the LNCaP cell line derived from a metastatic lesion of human prostatic adenocarcinoma. High-affinity specific androgen receptors were found in the cytosol and nuclear fractions of these cells [34]. These cells express PSA, their growth is inhibited by androgen withdrawal [35].

PC-3 cells do not express androgen receptor and PSA. Their proliferation is independent of androgens [36].

DU-145 are hormone-independent cells. This cell line derived from a human prostate adenocarcinoma metastatic to the brain [36].

The spherical nanoparticles contained poly(epsilon-caprolactone) (PCL) and a blend of poly(d,l-lactic-co-glycolic acid)-poly(ethylene glycol) conjugate(PLGA-PEG-COOH) and showed a mean diameter of about 150 nm. The encapsulation efficiencies ranged from 74% to 98%. The in vitro release tests were performed at a pH of 1.2 for 2 hours followed by a pH of 7.4 for 5 hours to simulate the conditions in the stomach and the intestine. After the first 2 hours in the acidic medium about 55% of resveratrol was released, during the following 5 hours the rest of resveratrol was released. Furthermore, the tests were performed at pH 6.5 and 7.4 for 24 hours in order to mimic the acidic microenvironment present in most tumours and physiological conditions, respectively. Under these conditions, 55% of resveratrol was released within 7 h, after 24 hours a complete release was found.

The authors showed by confocal fluorescence microscopy observations that the prostate cancer cells efficiently took up the nanoparticles. In all three cell lines, the cytotoxicity of the loaded nanoparticles (at all tested concentrations from 10 μM to 40 μM) was significantly bigger in comparison to that of free resveratrol. The calculated inhibition concentration to reduce cell viability of 50% (IC50) of the loaded nanoparticles amounted to 16 μM, 18 μM and 35, 3 μM for DU-145, LNCaP and PC-3 cells, respectively. Concerning free resveratrol, the IC50 values amounted to 28, 51 and 47 μM for DU-145, LNCaP and PC-3 cells, respectively. At a concentration of 20 μM the loaded nanoparticles showed an increased growth inhibition of DU-145, PC-3 and LNCaP cells of 69, 40 and 23% respectively. Nanoparticles which were not loaded with resveratrol showed no significant effect. The enhanced effects of the nanoparticles compared to free resveratrol may be explained by endocytosis of the nanoparticle by the tumour cells leading to an intracellular release of resveratrol.

Effects of resveratrol and curcumin encapsulated in alginate nanoparticles on the prostate cancer cell line DU145

In the following study by Saralkar and Dash [37], the effects of two different natural substances, curcumin and resveratrol, were examined at the prostate cancer cell line DU145 using calcium alginate nanoparticles for encapsulation. The yellow substance curcumin can be obtained from the rhizomes of Curcuma

longa (better known as the spice turmeric) and exhibits anti-cancer effects, too [38].

The particle size of the nanosuspension and freeze-dried nanoparticles amounted to 12.5±1.1 and 60.2±15 nm, respectively. After 24 hours 88% of resveratrol and 16% of curcumin was released, respectively. In their preparation, the authors found a poor cellular uptake for resveratrol from either solution or nanoparticles. Possible explanations are a rapidly occurring efflux of resveratrol out of the prostate cancer cells or the presence of serum proteins in the used medium, which may lead to binding of resveratrol. Cytotoxic effects of the drug-loaded nanoparticles on DU145 cells were found and differed significantly from the blank nanoparticles. However, since a combination of two substances were used and due to the poor cellular uptake of resveratrol, the precise cytotoxic effect of resveratrol alone cannot clearly be indicated in this in vitro study. The toxicity of the nanoparticles was smaller compared to the drug solution at high concentrations. In order to provide an indication, if an intravenous administration of the nanoparticles would be safe, hemolytic studies on human blood cells were performed. These studies showed nearly no hemolysis for blank and drug-loaded nanoparticles.

Effects of different resveratrol loaded nanoparticles on the prostate cancer cell line PC-3

In the in vitro study of Eroglu [39] nanoparticles containing resveratrol were shown to be cytotoxic against PC-3 cells in vitro with a good drug delivery efficiency. But in this study a very special type of nanoparticle was developed: The authors used a poly(2-hydroxyethyl methacrylate) core, in which resveratrol was encapsulated, and coated these nanospheres with the cationic polymer chitosan.

Chitosan is naturally found in the hard outer skeleton of shellfish like shrimp, lobster, and crabs. Amongst others, it is used for delivering anticancer drugs to tumour cells. Loaded nanoparticles with a surface modified by chitosan were shown to be more stable, permeable and bioactive [36]. It seems to be a good solution if no additional cytotoxic effect of components of the nanoparticle is wanted.

The following working group investigated again the effects of resveratrol loaded nanoparticles on the prostate cancer cell line PC-3, but used a totally different nanoparticle containing gold, which by itself reveals cytotoxic effects.

Thipe et al. [41] investigated cytotoxic effects of these nanoparticles not only on a prostate cancer cell line in vitro, but also on a breast cancer cell line (MDAMB-231), and a pancreatic cancer cell line (PANC-1) with promising results for all of them. Gold nanoparticles (Au-NPs) exhibit pro-apoptotic properties. For the synthesis of resveratrol-conjugated gold nanoparticles (Res-Au-NPs) trans-resveratrol was used to reduce Au3+ to Au0 at room temperature and by this way conjugated onto the surface of the Au-NPs. Further investigations were performed with four different types of nanoparticles: conventional resveratrol-conjugated gold nanoparticles (Res-Au-NPs), resveratrol-conjugated gold nanoparticles encapsulated by gum arabic (GA) (Res-GA-Au-NPs). By this way, the overall stability of the particles was increased. Additionally, two further nanoparticles were designed using the 3fold amount of resveratrol in the reaction mixture without and with gum arabic called 3x Res-Au-NPs and 3x Res-GA-Au-NPs, respectively. In these nanoparticles the number of trans-resveratrol conjugated molecules on the surface of the Au-NPs was increased. Stability studies showed a very good stability of the Res-Au-NPs and Res-GA-Au-NPs after 24 hours. Both types of nanoparticles were efficiently internalized into the tumour cells within 2 hours, an optimum cellular uptake was demonstrated between 4 and 24 hours of treatment. The four different nanoparticles were tested in cell viability studies on the three different cancer cell lines. Additionally, free resveratrol was used. The anti-tumour efficacy was concentration dependent. The nanoparticles with a threefold resveratrol corona were significantly more effective compared to Res-Au-NPs and Res-GA-Au-NPs, respectively. The effectiveness of free resveratrol molecules was comparable to Res-Au-NPs and Res-GA-Au-NPs. In all tested cell lines, both nanoparticles with threefold resveratrol content (3x Res-Au-NPs and 3x Res-GA-Au-NPs) showed the highest efficacy.

Further studies are necessary to investigate the applicability of such nanoparticles in vivo.

The following study emphasized the meaning of surface functionality for the effectiveness of resveratrol-loaded nanoparticles. Furthermore,

synergistic effects of resveratrol and docetaxel on the prostate cancer cell line PC3 was proven.

Chaudhary et al. [42] investigated the synergistic effects of nanoparticles loaded with resveratrol and docetaxel in vitro, also mimicking drug-resistance in the used prostate cancer cell line by hypoxic conditions. For that purpose, different types of nanoparticles were created and investigated. The authors developed uniformly sized mesoporous silica nanoparticles (MSNs) with an average size of about 60 nm. The surface of these nanoparticles was changed in two different ways: One group of MSNs were modified by adding negatively charged phosphonate groups (PO3-MSN), the other group of MSNs were modified by adding positively charged amine groups (NH2-MSN). A variety of in vitro examinations was performed using these modified MSNs loaded with resveratrol and MSNs without encapsulated resveratrol were utilised as control. In other experiments free resveratrol was tested, additionally, in comparison to the resveratrol loaded MSNs to show possible differences in the anti-proliferative/cytotoxic effects on the prostate cancer cell line PC3. The pore size of the MSNs amounted to 2.7 nm. At a pH of 7.4 free resveratrol and NH2-MSNs loaded with resveratrol showed a release of the majority of RES in the first 8 hours. PO3-MSNs showed a significantly slower release of resveratrol: At pH 7.4 40% of the drug was released during the first 8 hours, 65% during the first 24 hours. These experiments were also performed at a pH of 5.5, which should mimic the pH inside a cancer cell. Under acid conditions, both the PO3-MSNs and NH2-MSNs showed a release of 40% over 24 hours, whereas 60% of the non-encapsulated RES was released in 24 hours. Resveratrol inhibited the proliferation of the PC3 cells dose-dependently. The IC50 value of free resveratrol was calculated to be 14.86 μM. The phosphonate modified MSNs significantly enhanced the anti-proliferative potential of RES: Their IC50 amounted to 7.15 μM. The amine modified MSNs didn't affect the proliferation, their IC50 value amounted to 20.45 μM. These results suggest that the PO3-MSNs are mostly suitable for using them under clinical conditions. Another goal of this study was to examine, if resveratrol, encapsulated in MSNs, could reduce the resistance of the prostate cancer cells to docetaxel. Resistance against taxanes is influenced by the tumour microenvironment [43]. Hypoxia plays an important role for the development of resistance against substances interacting with the cell cycle [43]. Therefore, in this study resveratrol at a low concentration of 10 μM was combined with docetaxel used at various concentrations from 0.1 to 100 nm and the effects of these combinations and additionally of Docetaxel alone on the cell viability of PC3 cells were examined under normoxic and under hypoxic conditions. Docetaxel alone was more cytotoxic under normoxia compared to hypoxia. By using the combination of docetaxel with resveratrol alone or with PO3-MSNs encapsulating resveratrol the cell viability of PC3 cells was significantly reduced under normoxic and under hypoxic conditions. Even in the lower used concentrations of docetaxel of 0.1, 1 and 10 nm, respectively, the cancer cell burden was reduced to half after treatment with the combination of docetaxel and resveratrol or docetaxel and resveratrol loaded PO3-MSNs, respectively. Between both combinations no significant difference was found at all concentrations of docetaxel under hypoxic and normoxic conditions. These results suggest a synergistic effects of docetaxel and resveratrol even under hypoxic conditions, mimicking an aspect of chemoresistance of prostate cancer cells. They also suggest no reduction of the anti-proliferative activity of resveratrol when used in an encapsulated form as in this study as a nanoparticle.

RES and other polyphenols, especially used in combination with well-known anti-cancer drugs, may represent the next generation of anticancer substances for treating drug resistant prostate cancer. Experiments on docetaxel-resistant prostate cancer cells could confirm this idea. The following passage summarizes and discusses such an in vitro study including experiments on a docetaxel-resistant prostate cancer cell line and also focuses on an appropriate target for the nanoparticles found in the cell membrane of the prostate cancer cells: the folate receptor.

Overcoming drug resistance of prostate cancer cells by using the folate receptor as binding site for loaded nanoparticles

In a very interesting study by Singh et al. [44] the folate receptor was used as a target for specially created nanoparticles loaded with

resveratrol alone or in combination with docetaxel. The folate receptor is known to be expressed in different cancer types including prostate cancer. The authors performed different types of experiments. In one more clinical investigation the expression of the folate receptor was investigated using prostate cancer tissue of 80 patients suffering from prostate cancer of different histological differentiations. Stage IV cancer (poorly differentiated) was diagnosed in 39 cases. In this subgroup the expression of folate receptor alpha was comparatively higher than in moderately differentiated carcinomas. Furthermore, in five different cell lines of prostate cancer the expression of folate receptor alpha was determined. The docetaxel-resistant PC3-R cell line showed a higher expression of folate receptor alpha in comparison to the non-resistant PC3 cell line. These data suggest that this receptor could be used as a potential target for specially developed nanoparticles conjugated with folate.

Based on these ideas, the authors developed novel planetary ball milled nanoparticles (NPs) encapsulated with resveratrol alone or with a combination of docetaxel and resveratrol. These nanoparticles were conjugated with folic acid on the surface. The IC50 values of the free drugs docetaxel and resveratrol in the resistant cell line PC3-R amounted to 280 nm and 120 μM, respectively, showing a high resistance. These values were 28fold and 4fold higher compared to those determined in the parental non-resistant PC3 cells. The IC50 values of docetaxel and resveratrol increased clearly and amounted to 10 nm and 3 μM in the PC3-R cell line, respectively, when the substances were encapsulated in the planetary ball milled nanoparticles conjugated with folate. Further analysis showed a significant increase in the number of apoptotic cells after treatment with nanoparticles encapsulating resveratrol alone and a much stronger increase in the number of apoptotic cells after treatment with nanoparticles encapsulation the combination of the two drugs. In PC3-R cells the early and late apoptotic cells were increased by 47% and 19% when treated with the nanoparticles encapsulating both substances in concentrations of 3 μM for resveratrol and 0.01 μM for docetaxel. Using the encapsulated resveratrol alone the percentage of apoptotic cells amounted to 21% (early apoptotic) and nearly 10% (late apoptotic). The control in which empty nanoparticles were used showed 9% of apoptotic cells (early and late). In the non-resistant cell line PC3 the treatment with nanoparticles containing the combination led to 51% of apoptosis (early and late apoptosis) and to 21% (early and late apoptosis) using the encapsulated resveratrol alone. Regarding the values of encapsulated resveratrol alone, these results showed a similar effect of encapsulated resveratrol on the apoptosis in the non-resistant and resistant cell line with a higher number of apoptotic cells in the resistant cell line.

Furthermore, the synergistic apoptotic effect of both substances on the resistant prostate cancer cell line, was shown, too. The percentage of apoptotic cells was higher than in the non-resistant cancer cell line. This even stronger effect may be due to the higher number of folate receptors in the resistant cell line. Additional experiments showed that in resistant PC3-R cells treated with the nanoparticles containing both substances for 48 hours, the expressions of pro-apoptotic and anti-apoptotic markers were altered: A down-regulation of the anti-apoptotic markers BCL-2 and survivin occurred and an upregulation of the pro-apoptotic markers BAX, BAK and cleaved caspase 3 was shown. The treated cells were additionally analysed for the expression of NF-kB p65 known for contributing to cell survival as well as progression and proliferation of tumour cells. Its expression was downregulated after the combined treatment. Singh et al. [44] found an increased expression level of ABC-transporter markers like ABCB1, ABCC1, ABCG2, in the docetaxel resistant cell line PC3-R compared to PC3. After treatment of the cells for 48 hours with the two types of nanoparticles containing resveratrol alone or the combination of resveratrol with docetaxel the expression levels of the marker genes were reversed. By this way, this treatment act as inhibitor of the drug efflux and enhances the intracellular concentration of the drugs inside the former resistant prostate cancer cell line. All results of this study suggest that the specially generated planetary ball milled nanoparticles loaded with resveratrol alone (or, better, with a combination of docetaxel and resveratrol) conjugated with folic acid on the surface, may represent an effective anticancer treatment for docetaxel-resistant prostate cancer. Further clinical studies are neces-

sary to confirm the clinical effectiveness in vivo.

In vivo effect of resveratrol in combination with curcumin on prostate cancer in PTEN knockout mice

Narayanan et al. [45] created a liposome encapsulation of resveratrol combined with curcumin and investigated the effects of this combination on prostate cancer in PTEN knockout mice. Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) represents one of the most frequently disrupted tumour suppressors in different types of cancer [46] like prostate cancer [47]. It was first identified in 1997 as PTEN/MMAC1 gene (mutated in multiple advanced cancers) [47, 48]. The authors investigated the effects of liposome encapsulated resveratrol combined with curcumin in prostate-specific PTEN knockout mice. This combination significantly decreased the prostatic adenocarcinoma in vivo. In the group treated with the combination a significant decrease of the prostate weight was found as well as histological changes suggesting a decrease of the tumour growth. The total number of adenocarcinomas determined in the group which received the combined treatment was significantly reduced from 400 to 110. Pure liposomes without both substances had no effect. Additional in vitro studies showed that the combination of both substances inhibited the cell growth and induced apoptosis. A more than 5-fold increase of the rate of apoptosis was shown in cells treated with this combination. The cell cycle analysis of those cells exposed to resveratrol in combination with curcumin (5 μM of each substance) exhibited a G1 peak associated with a distinct pre-G1-peak being a sign of apoptotic cells. Furthermore, a significant inhibitory effect of resveratrol combined with curcumin on p-Akt (ser 473), androgen receptor, cyclin D1 and mTOR (mammalian target of rapamycin) proteins was shown in the prostate cancer cell line PTEN-CaP8. The results of this interesting study corroborate the assumption that resveratrol, optionally in combination with other substances, may reduce the incidence of prostate cancer due to the loss of the tumour suppressor gene PTEN. Further in vivo studies including resveratrol as monotherapy are necessary to further the research in this promising field.

Conclusion

The studies clearly indicate that resveratrol-loaded nanoparticles exhibited in vitro a remarkable anti-cancer activity in various hormone-sensitive and -insensitive prostate cancer cell lines including docetaxel-resistant prostate-cancer cells. The used types of nanoparticles varied and influenced the outcome. Additionally, the meaning of the surface functionality for the effectiveness of resveratrol-loaded nanoparticles is emphasized.

No reduction of the anti-proliferative activity of resveratrol was shown when used in an encapsulated form, in some studies even an enhanced effect of the nanoparticles compared to free resveratrol was detected.

Additionally, synergistic effects of resveratrol and docetaxel on the prostate cancer cell line PC3 were proven.

Promising data suggested that the folate receptor could be used as a potential target for specially developed nanoparticles conjugated with folate.

The combination of encapsulated resveratrol combined with curcumin significantly decreased the prostatic adenocarcinoma in vivo.

Shortcomings of the studies are that they often only focus on one cell line.

In studies using cancer cell lines the effects of the used drugs on other tissues cannot be demonstrated. Therefore, conclusions about possible side effects cannot be drawn. Another shortcoming of the studies using a combination of resveratrol loaded nanoparticles with another substance is that it is not clear which of the substance led to the described effect on the tumour cells.

RES and other polyphenols, especially used in combination with well-known anti-cancer drugs like docetaxel or paclitaxel or used in combination with other phytochemicals, may represent the next generation of anticancer substances for treating drug resistant prostate cancer. The researchers have done pioneering work in this field. However, future research is needed, especially in vivo and clinical studies are needed to confirm the clinical effectiveness of resveratrol-

loaded nanoparticles as complementary treatment option for the treatment of prostate cancer. In future studies different types of prostate cancer should be investigated separately. Additionally, the effects of the single substance resveratrol without any combined agent on prostate cancer should be examined before examining combination therapies.

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None.

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