PH. D. THESIS

INVESTIGATION OF ANTI-CANCER ACTIVITY OF METAL NANOPARTICLES AND HISTONE-DEACETYLASE INHIBITORS

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Introduction

Chemo- and radiotherapy are the most commonly employed treatment modalities for malignant diseases. Besides the conventionally applied anti-cancer agents, novel compounds suitable for personalized and targeted therapeutic approaches and antibody-based treatment regimens have gathered grounds. In radiotherapy, typically high energy irradiation is applied to the tumor tissue to cause damages to the membrane structures and various macromolecules such as the DNA of tumor cells, however, ionizing radiation can harm healthy tissues and organs surrounding the tumor. These adverse effects can be reduced and at the same time the efficacy of the treatment might be enhanced by the application of radiosensitizing agents.

The potential and the applicability of metal nanoparticles in oncotherapy have been investigated intensively in recent years due to the unique physical and chemical properties of these nanomaterials and their special interactions and reactivity with biological systems. Although silver nanoparticles (AgNPs) are the most commonly utilized nanomaterials, this is mostly due to their anti-microbial activity, however, the intrinsic anti-tumor effects of these materials have been highlighted in the last decade. AgNPs induce oxidative stress and cause apoptosis in tumor cells via the release of reactive silver ions. On the other

hand, gold nanoparticles (AuNPs) are considered as biologically inert particles, nevertheless, they hold enormous potential in drug delivery, photothermic therapy, and as radiosensitizer agents, since they are capable to increase the physical effect of ionizing radiation by the generation of reactive electrons due to Auger effect and Compton scattering. The rationale for utilizing nanoparticles in tumor therapy is also based on the enhanced permeability and retention effect, where the leaky vasculature of the tumor promotes the passive accumulation of nanometer-sized materials in the tumor tissue.

The increased activity and the overexpression of histone deacetylase (HDAC) enzymes were observed in several tumor types, therefore, the possible application of inhibitors acting on these enzymes in tumor therapy immediately emerged. The anti-cancer activity of HDAC inhibitors was later proven in hematopoietic malignancies and solid tumors as well. HDAC inhibitors influence the acetylation level of histone and non-histone proteins and induce programmed cell death via activating different apoptotic pathways. Acetylation of histone proteins results in a relaxed chromatin structure, which makes the DNA more vulnerable to various genotoxic factors, such as ionizing radiation, reactive electrons or oxygen species. Based on these facts, HDAC inhibitors in combination with metal

nanoparticles and radiation therapy can increase the specificity and effectivity of cancer therapy.

Aims

Our aim was to investigate the anti-cancer efficiency of the combined application of metal nanoparticles and HDAC inhibitors in two experimental setups.

1. In the first study the cellular events and the anti-cancer performance of AgNPs and the HDAC inhibitor Trichostatin A (TSA) were investigated *in vitro*.
2. In the second experimental approach the combinational radiosensitizing activity of AuNPs and the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) was studied in 2D and 3D cancer cell cultures.

Materials and methods

The cellular uptake of the metal nanoparticles was detected by transmission electron microscopy. The consequences of HDAC inhibition by applying TSA or SAHA alone or in combination with metal nanoparticles were determined by acetylated-lysine-specific immunocytochemistry and by western blotting.

The viability of cells upon AgNP and TSA treatments was investigated by MTT assay. The synergistic interaction between AgNPs and TSA treatments was determined by CompuSyn software utilizing the results of the MTT assays.

The amount of reactive oxygen species was detected by DCFDA staining, the DNA damaging effect, and the apoptosis-inducing activity of AgNPs and TSA were determined by γH2AX and cleaved caspase-3 immunocytochemistry, respectively.

The synergistic radiosensitizing activity of AuNPs and the HDAC inhibitor SAHA on 2D cancer cell cultures was investigated by MTT and colonyforming assays and the synergistic interaction between the two agents was determined by using the CompuSyn software. The amount of DNA double strand breaks caused in cancer cells by the ionizing radiation following AuNPs and SAHA treatment was assessed by γH2AX immunostaining. The synergistic radiosensitizing effect of AuNPs and SAHA was verified on 3D cancer cell spheroids as well. For this, DNA double strand breaks, induced in cancer cells of the 3D culture, were vizualized by γH2AX immunohistochemistry and the colonyforming propensity of AuNP- and SAHA-treated irradiated cells was investigated by clonogenic assay.

Major findings

1. Metal nanoparticles are internalized by tumor cells and are accumulated in the cytoplasm, most often in multivesicular bodies.
2. The intracellular presence of metal nanoparticles does not influence the HDAC inhibitory effect of TSA or SAHA, when these inhibitors are applied together with metal nanoparticles.
3. AgNPs and the HDAC inhibitor TSA synergistically decrease the viability of tumor cells.
4. As a result of the combined application of AgNPs and TSA the level of reactive oxygen species, the number of DNA double-strand breaks, and the amount of apoptotic cells significantly increase compared to the untreated samples and to the individual AgNP or TSA treatments.
5. The combination of AuNPs and the HDAC inhibitor SAHA synergistically decreases the viability and the colonyforming capacity of tumor cells after ionizing radiation.
6. The number of DNA double-strand breaks caused by ionizing radiation is significantly higher in cancer cells receiving AuNP and SAHA together than in cells exposed either to AuNPs or to SAHA.

7. The enhanced radiosensitizing feature of the AuNP and SAHA combination is expressed on 3D cancer cell spheroids as well, since these cancer cells exhibited significantly decreased colonyforming capacity and increased amounts of DNA damage after irradiation, compared to individual AuNP- or SAHA-treated samples.
8. Inhibition of HDACs by TSA or SAHA results in a relaxed chromatin structure, which renders the DNA more vulnerable to the damaging actions of the ionizing radiation, enhanced by the presence of AuNPs, or of the cytotoxic effects of AgNPs.

Summary

According to our results, we can conclude that the combination of both AgNP+TSA and AuNP+SAHA, the latter with ionizing radiation, synergistically decrease the viability of tumor cells. AgNPs in combination with TSA significantly increase the quantity of reactive oxygen species, the amount of DNA double-strand breaks and the number of apoptotic cells compared to the untreated and to the AgNP- or TSA-treated samples.

The combination of AuNPs and SAHA increases significantly the DNA damaging effect of ionizing radiation and

decreases the colonyforming capability of irradiated tumor cells growing in 2D and 3D cultures, compared to untreated and to AuNP-, or SAHA-treated cells.

In both studies we have shown that HDAC inhibitors assist the formation of an opened chromatin structure, which makes the genetic material of the cells more vulnerable to harmful genotoxic agents. In combinational treatments, where metal nanoparticles are also applied, these harmful factors can be the AgNP-triggered reactive oxygen species or a multitude of reactive electrons, generated by the interaction of AuNPs and ionizing radiation. In our study, we proved the synergistic anti-cancer activity of AgNP+TSA and the radiosensitizing effect of AuNP+SAHA combinations.

List of publications

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Publications that form the basis of the PH.D thesis:

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