Review

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Novel radioisotope-based nanomedical approaches

Abstract: Radioisotope therapy of cancer is on the rise applying mainly β -emitting radionuclides. However, due to exposure of healthy tissues, the maximum achievable radiation dose with these is limited. Auger-electron emitters (AEs) represent a promising alternative because of their mode of decay within a short nanometer range. The challenge is that their therapeutic efficacy relies on a close vicinity to DNA. To overcome this and to minimize toxicity, the construction of smart nanomedical devices is required, which ascertain tumor cell targeting with succeeding cellular uptake and nuclear translocation. In this review we describe the potential of AEs with focus on their delivery down to the DNA level and their cellular effects. Reported efforts comprise different tumor-targeting strategies, including the use of antibodies or peptides with nuclear localizing sequences. Recently, attention has shifted to various nanoparticle formats for overcoming delivery problems. To this end, these approaches have mostly been tested in cell lines in vitro applying AEs more suited for imaging than therapy. This defines a demand for nanomedical formulations with documented in vivo activity, using AEs selected for their therapeutic potential to come closer to real clinical settings.

Keywords: Auger-electron emitters; DNA damage; nuclear targeting; radionuclide therapy; tumor targeting.

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Introduction

α -, β - or Auger-electron emission from radionuclides can be used to treat cancer by irradiating and killing tumor cells in targeted radionuclide therapy. Currently approved radiopharmaceuticals include a group of agents that specifically accumulate in a given target tissue, e.g., ^{131}I , which accumulates in thyroid tissue (1) or antibodies that target cell surface molecules found on tumor cells. The latter include, for example, Zevalin and Bexxar, which are radio-labeled antibodies against CD20 on B-cells in non-Hodgkin lymphoma labeled with ^{90}Y and ^{131}I , respectively (2).

The use of radionuclides for cancer therapy has been suggested already back in 1938 (3), and has been put into practice in benign and malignant thyroid disease in 1943 and 1946, respectively, applying the β -emitter ^{131}I , which is still in use for this purpose (1, 4). Since iodine is part of the thyroid hormone it is absorbed from the digestive tract and transported by the blood to be extracted by and organically bound in the gland, thus reducing radiation to non-target organs. In theory, ^{131}I appears to be an ideal clinical radioisotope, but in reality, things are more complicated and even if radioiodine therapy of thyroid diseases has been a tremendous clinical success (5–8), the approach needs optimization and is not directly applicable in other types of cancer.

Peptide receptor radionuclide therapy (PRRT) of neuroendocrine tumors applies β -emitters ^{177}Lu or ^{90}Y for irradiation, an approach that has proved effective in tumors with a high concentration of somatostatin

receptors (SSTRs) because the radioisotopes in this setting are mainly absorbed by the tumors or excreted by the kidneys (9, 10). Unfortunately, although helpful, this kind of therapy is not curative, probably because the risk of kidney and bone marrow damage limits the maximum achievable dose (9). Furthermore, β -emitters rely on cross-fire effects from surrounding labeled cells in order to increase the radiation dose to the tumor but this effect is insignificant for micro-metastases or single tumor cells. Thus, there is a risk that these tumor cells receive a too low radiation dose to be effectively killed.

Therefore, other isotopes with better radiation characteristics and with fewer side effects have been matter of intense searches. The potential of low-energy Auger electrons in cancer therapy has been suggested some decades ago, when their ability to induce complex and severe damage to DNA was discovered (11). However, due to their short-range emission, it has become clear that Auger electron emitter-based radiotherapy requires adequate tumor delivery, high nuclear accumulation and retention as well as that the radionuclides need to be in close proximity to DNA to have therapeutic value. This review will focus on recent developments in tumor-targeting, internalization and nuclear translocation/DNA binding that have been made with respect to the use of Auger-electron emitters (AEs) in the treatment of cancer.

AEs in targeted radionuclide therapy

Conventionally, isotopes emitting long-range β -particles are used for radionuclide therapy of cancer (12), but in recent years encouraging results have also been obtained with radionuclides emitting low-energy electrons, e.g., Auger- and conversion electrons (13–19).

Auger electrons (here used as the collective name for Auger, Coster-Kronig and super Coster-Kronig electrons) are low-energy orbital electrons emitted from the atomic shells of the decaying nuclide. They are created as a result of atomic de-excitation following the creation of an electron vacancy (hole) in an electron capture (EC) decay or internal conversion (IC) process. In the de-excitation process, the vacancy is rapidly filled with an electron from a higher energy state and the remaining energy is then either emitted as a characteristic X-ray photon or an Auger electron. In the latter case, an additional vacancy is created (Figure 1). The de-excitation process is then repeated so the vacancies are moved towards the outermost shell and thus, a cascade of Auger electrons is created. On average, 5–30 electrons with energies ranging from a few eV to some keV, are emitted from a nuclide undergoing an EC decay or an IC process (12).

The high biological toxicity and the considerable therapeutic potential of these low-energy electron-emitters are mainly associated with the very high ionization density created in biological tissue (high-LET-like effect) from their decay (12, 15). This is a consequence of the emission of this electron cascade in each radioactive decay, with all electrons having low energies and thus, resulting short ranges in biological tissue.

Consequently, the decay leads to a highly localized energy deposition in the vicinity of the decay site (Figure 2), corresponding to an extremely high and local radiation dose. Further, as a consequence of the short range, it is conceptually possible to minimize the undesirable radiation dose to the normal tissue and resulting side effects, by ensuring that the radionuclides are not internalized into the healthy cells, which might be achieved by the use of appropriate targeting mechanisms and devices (14, 15, 20, 21). It has thus been proposed that it should be possible to administer a large amount of radioactivity of these isotopes to a patient to increase the therapeutic

Figure 1 Illustration of the Auger-process. When the initial vacancy is created from the EC-decay or the IC-process, electronic transitions lead to the emission of characteristic X-rays and/or Auger-electrons.

Figure 2 The spatial ionization (*) patterns associated with α -, β - and Auger decays. In the vicinity of the Auger-decay site, a very high ionization density is created giving rise to the high-LET effects also observed with α -particles.

effect, while keeping the side effects in the critical organs at a minimum (22). The short-range radiation potentially makes this type of radionuclides particularly suited for therapy of small metastases and disseminated cancer cells, owing to the minimal irradiation the surrounding, normal tissue would be exposed to, in contrast to the application of conventional β -particle emitters, which currently are undergoing clinical trials (17, 21, 23).

Due to the electron-ranges of AEs that typically are less than one cell diameter, it is important that the radionuclides are internalized into the cancer cells – and, preferably, into the cell nuclei in close vicinity to the DNA to maximally exploit this biological effect (12, 13, 15, 17, 18). If the decaying nuclides are located in the cell nucleus, close to the DNA, high-LET-like effects are observed (the so-called “Auger effect”). Effects resembling the effects observed with α -particles, resulting in a survival curve with no shoulder and Relative Biological Effectiveness (RBE) values of up to approximately 9 (24). On the other hand, if the decay takes place in the cytoplasm, at the cell surface or in the extracellular space, the effects resemble those observed with low-LET radiation e.g., X-rays or β -particles with a pronounced shoulder on the survival curve (20, 24–26).

The therapeutic effectiveness of AEs, once inside the nucleus, occurs mainly through extensive DNA damage that is difficult to repair because the most deleterious forms of DNA damage; clusters of DNA double strand breaks (DSBs) are caused. This was highlighted in recent experiments using ^{125}I attached to a DNA intercalator, which revealed that the number of DNA DSBs decreases

as the ^{125}I atom is located farther away from the DNA. The scavenger dimethyl sulfoxide (DMSO) did not affect the DNA DSB yield when the distance between DNA and ^{125}I was below 1,2 nm supporting a complete direct effect. However, above 1,2 nm, no DSBs were observed in the presence of DMSO supporting completely indirect effects (27).

Delivery to target cells – carriers for AEs

Currently, delivery of AEs in vivo faces a number of challenges, including the delivery vehicle, tumor markers to ensure selective targeting, and nuclear translocation and DNA targeting functionalities (Figure 3). Tumor targeting has focused on the use of antibodies (28, 29), peptides, which serve as ligands for cell surface receptors (14, 30), and nanoparticles (31).

Increased nuclear targeting has generally been attempted to be optimized using a nuclear localization sequence (NLS), most often from the SV40 Large T-antigen. The NLS is recognized by the importin α/β heterodimer which shuttles the cargo across the nuclear pore complex (32). Though, no DNA binding occurs via this technique and hence, no high-LET-effects, the radiation dose to the DNA increases by increasing the amount of activity located in the nuclei of the targeted tumor cells. Nuclear targeting has also been analyzed using nucleosides, oligonucleotides, DNA intercalators, hormone receptor ligands, which associate with DNA or peptide ligands that target a cell surface receptor, which then in turn itself translocates to the nucleus (33).

Antibodies

Antibodies used for delivery of AEs to cancer cells include antibodies against cell surface proteins, particular in hematological malignancies such as CD74 (34–36) or CD33. When used in combination with ^{111}In and a nuclear localization sequence the antibodies caused localization of ^{111}In to the nucleus of drug-resistant AML (acute myeloid leukemia) cell lines and primary tumor cells and decreased clonogenic survival (18, 37).

Several groups have also focused on antibodies targeting the epidermal growth factor receptor (EGFR) or human epidermal growth factor receptor 2 (HER2), which are frequently overexpressed in cancer (38, 39). ^{111}In -nimotuzumab and ^{111}In -NLS-nimotuzumab were

Figure 3 Schematic drawing showing different methods for targeting the AEs to the tumor cell, the nucleus and the DNA. Once binding occurs of (A) an antibody- or (B) a peptide-conjugate to their cognate cell surface receptors, the conjugate is internalized. However, only if the conjugate is modified by a nuclear localization sequence (NLS) are the AEs efficiently transported to the nucleus via the importin α/β complex, leading to nuclear accumulation of the AEs. (C) Nanocarriers, here exemplified by a liposome loaded with AEs conjugated to a DNA intercalator. The particles are targeted to the tumor cells via an antibody conjugated to nanocarrier surface. Once inside the cell, the AE-intercalator translocates to the nucleus and binds to DNA. When the AEs are in very close proximity to DNA, they can induce highly toxic DNA DSBs.

both internalized by EGFR overexpressing breast cancer cells. ^{111}In -NLS-nimotuzumab quickly accumulated in the nucleus, induced more DNA damage and consequently was more effective in reducing clonogenic survival in vitro. However, in vivo the NLS-conjugate had a lower tumor uptake than the conjugate without NLS (40).

Similarly, the anti-HER2 antibody trastuzumab modified with NLS-peptides and conjugated to ^{111}In was internalized in breast cancer cells, efficiently accumulated in the nucleus and was more cytotoxic compared to the radio-labeled antibody without NLS. In contrast to the results with nimotuzumab, there was an increased nuclear uptake of ^{111}In -NLS-trastuzumab in HER2-positive tumor xenografts and the mice had a significantly longer survival

time compared to mice treated with unlabeled antibody. Furthermore, the addition of NLS peptides did not alter the biodistribution of the immunoconjugate (19, 41).

As described above, the introduction of a nuclear localization sequence might affect the in vivo behavior. For example, when an unspecific antibody, labeled with ^{111}In , was conjugated to the cell penetrating peptide tat (transactivator of transcription), which also harbors an NLS, it increased blood elimination and increased nuclear accumulation not only in breast cancer xenografts but also in liver and kidney cells (42). This emphasizes the complexity of changes introduced by additional functionalities and defines a demand for platform nanomedical approaches, which result in more predictable behavior.

Peptides

Several malignant tumors overexpress cell surface receptors, which can be targeted with radiolabeled receptor-specific peptides. Much attention has been paid to peptides that bind the somatostatin receptor which is, for example, overexpressed in neuroendocrine tumors (43).

Inclusion of an NLS at the N-terminal part of the somatostatin analogue peptide DOTATOC (DOTA-[Tyr³]-octreotide) labeled with ¹¹¹In, increased cell retention and increased nuclear accumulation of the radioisotope, whereas placing the NLS at the C-terminal part of the peptide had no effect or even decreased internalization compared to unmodified ¹¹¹In-DOTATOC (44). However, recent results from our group (unpublished) indicate that liver uptake is strongly increased in vivo in tumor-bearing mice by inclusion of the NLS at the N-terminal of ¹¹¹In-DOTATOC compared to unmodified radioligand. More recently, an analogue of the DNA intercalator Hoechst was conjugated to the somatostatin analogue TATE ([Tyr³, Thr⁸]-octreotide) and labeled with ¹²⁵I to form ¹²⁵I-Hoechst-TATE. The conjugate was internalized through an SSTR2-receptor-mediated mechanism, but only a small fraction of the internalized radioactivity accumulated in the nucleus, albeit a higher fraction compared to a complex without Hoechst (¹²⁵I-TATE). The labeled peptides both localized to human xenografts in mice, however, the addition of the Hoechst moiety resulted in a larger proportion of radioactivity localizing to the liver as compared to ¹²⁵I-TATE alone, which predominantly was found in the kidneys (45).

Other surface receptors have also been used for targeting of peptide-ligands, including EGFR for breast cancer. Reilly et al. initially showed that EGF (epidermal growth factor) conjugated to ¹¹¹In was internalized in breast cancer cells overexpressing EGFR. Approximately 15% of the internalized radioactivity accumulated in the nucleus within 24 h. This resulted in a decreased growth rate and decreased cell survival. In vivo, no morphological changes were seen in either kidney or liver, the two organs, in which most of the radioactivity was distributed (14).

Specific binding of the F3 peptide from human high mobility group protein 2 (HMGN2), labeled with ¹¹¹In, to nucleolin on the surface of tumor cells led to internalization in breast cancer cells and translocation to the nucleus and nucleoli, causing DNA damage and reduced clonogenic survival in cells. Furthermore, in vivo the conjugate arrested tumor growth, though tumor uptake was modest and most of the radioactivity was found in the blood and the kidneys (46). The F3 peptide has also been radiolabeled with ¹²⁵I, which also internalized in vitro, albeit no biological effects were evaluated (47).

^{99m}Tc conjugated to bombesin binds gastrin-releasing peptide receptor (GRP-r), which is overexpressed on the surface of several types of cancer cells (48). To increase internalization and nuclear delivery, a tat sequence was included. The conjugate was internalized into prostate- and breast cancer cell lines and translocated to the nucleus, causing a significant decrease in cellular proliferation in vitro (49).

Nanoparticles

Nanoparticles are thought to passively target tumors due to the enhanced permeability and retention effect (EPR), which may result from the disorganized tumor vasculature [reviewed in (50)]. Accordingly, passively targeting nanoparticles were historically the first ones to be tested. Recent strategies, however, focused on modifying the nanoparticle surface by attaching tumor-targeting units such as antibody-fragments, peptides or small molecules, which further enhance accumulation in the tumor through active targeting mechanisms via molecular interactions (51–61).

So far nanomedical approaches have generated a large number of drug delivery systems for radio-isotope imaging, including, for example, liposomes (62–65), iron oxide (66–68), polymers (69), dendrimers (70) and carbon nanotubes (71). Here we focus on recent studies using nanoparticles for delivery of AEs to tumor cells for therapeutic purposes, although so far most of them have the drawback of only being tested in vitro.

Liu et al. constructed 3-component nanoparticles consisting of a biotinylated anti-tumor antibody (Herceptin or trastuzumab for tumor cell targeting), a biotinylated penetrating peptide (tat for internalization and nuclear translocation) and a biotinylated ¹¹¹In-labeled antisense oligonucleotide (for DNA binding) all held together by streptavidin. The ¹¹¹In-labeled oligonucleotide accumulated in the nucleus of HER2 overexpressing cells and was cytotoxic for the cells (51, 52), which shows the validity of the approach, albeit in vivo studies remain to be performed.

A so-called modular nanotransporter could also enhance the nuclear accumulation and cytotoxicity of ¹²⁵I in EGFR-overexpressing cell lines. The modular nanotransporters are recombinant multifunctional polypeptides, which ensure receptor binding and internalization (EGF), endosomal escape (DTox; a translocation domain from diphtheria toxin) and nuclear translocation (NLS) (53).

Multifunctional block copolymer micelles (BCMs, nanoassemblies of amphilic copolymers) were labeled

with ^{111}In and conjugated with EGF. Uptake and nuclear accumulation was compared to ^{111}In -EGF in breast cancer cell lines and although cellular uptake of the BCMs was lower than of ^{111}In -EGF, a fraction of ^{111}In -EGF-BCM accumulated in the nucleus and gave a six-fold inhibition of cell growth in vitro compared to non-treated cells (54). Sedlacek et al. described an approach, where ^{125}I was covalently bound to a DNA-intercalating derivative of ellipticine and conjugated to a polymer with a pH-controlled release of the ^{125}I -ellipticine in endosomes plus DNA targeting via the intercalator. In vitro experiments showed that this nanoparticle accumulated in the nucleus and was cytotoxic in a panel of cancer cell lines (55).

Recently, a two-step targeting method was described for transporting ^{125}I into the nucleus of cells using PEG (polyethylene glycol)-stabilized liposomes, called nuclisomes. The liposomes were conjugated to EGF and were loaded with ^{125}I -Comp1. Comp1 is an intercalating daunorubicin derivative, employed for DNA intercalation in this setting. The nuclisomes were taken up by human EGFR-overexpressing glioma cells and considerably delayed cell growth in vitro (56). In another study, the nuclisomes were conjugated with a targeting device for HER2 and these were specifically taken up by HER2-overexpressing cells in vitro and reduced cancer cell survival (57). Furthermore, when the nuclisomes targeted to HER2 were given to mice carrying human ovarian cancer xenografts, they accumulated in the tumors and there was a significant difference between treated and mock treated animals since more than 50% of the surviving mice were tumor-free. In addition, no macroscopic or microscopic radiotoxic side effects were observed in the mice (31, 57, 58) supporting the nuclisome approach in specific also in vivo and the feasibility of the AE-based radiotherapy in general.

Cellular responses to AEs

DNA double-strand breaks (DSBs) are highly deleterious and their formation is a major determinant of the cytotoxicity of radiation therapy as well as of several chemotherapeutic agents. An early event following the formation of DSBs is the phosphorylation of histone H2AX at serine 139 by the PI3K-like kinases [i.e., DNA-PK (DNA dependent protein kinase), ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related)], resulting in γH2AX (72). The formation of γH2AX foci is abundant, fast, and correlates with DSBs, making it a sensitive marker that can be used to analyze DSBs. Indeed, this is a widely used method when evaluating the therapeutic potential of AEs in vitro.

The assay is often performed in addition to an assay that measures cellular survival/cell death since this is the ultimate endpoint response after radiation. Cells can die via different mechanisms following radiation-induced DNA damage, with the most important mode for solid tumor cells being mitotic death, i.e., the result of aberrant mitosis and a delayed type of cell death (73). Other modes of cell death following radiation include apoptosis and autophagy or the cells can enter senescence – a permanent cell cycle arrest. Below we summarize the biological effects and cellular responses caused by AEs in vitro and in vivo as well as some of the so far known determinants.

Chromatin structure influences radiosensitivity to external irradiation (74), but also to internal radiation since fewer DNA DSBs are induced in supercoiled DNA than in nicked or relaxed DNA by the AE ^{125}I in vitro (75). ^{111}In -EGF induced more DNA damage and stronger reduction of clonogenic survival, when the cells had been pretreated with inhibitors of histone deacetylases to relax DNA. Similarly, condensation of the chromatin resulted in fewer γH2AX foci (76). Conjugation of ^{111}In to NLS and an antibody, which preferentially recognizes leukemia stem cells, reduced the viability of AML cells. This was associated with DNA DSBs as visualized by γH2AX foci (77). Conjugation of a fragment of vascular endothelial growth factor (VEGF) to ^{111}In induced DNA DSBs and was cytotoxic in porcine aortic endothelial cells overexpressing the Flt1-receptor. This effect was increased by introducing a nuclear localization sequence (30). The internalizing anti-EGFR (cytoplasmic localization) and the non-internalizing anti-CEA (carcinoembryonic antigen, cell surface bound), both conjugated with ^{125}I , induced mitotic death in human colon cancer cells (78).

Bystander effects

Part of the AE radiotoxicity has been shown in vitro and in vivo to be due to bystander effects, which occur when radio-damaged cells induce cell death in neighboring non-irradiated cells. However, bystander effects not only include cell death, but also DNA damage, genomic instability, transformation, invasion, proliferation and radio-adaptive responses (79). The mechanisms behind bystander effects remain to be completely established, but direct cellular communication through gap junctions and indirect mechanisms through the release of signaling molecules play important roles (79–81).

Bystander effects have mostly been described for external radiation; however several reports describe bystander effects induced by AE radionuclides both in

cell culture and in animal models. Medium from human glioma or bladder carcinoma cells (donor cells), incubated with ^{123}I -meta-iodobenzylguanidine (MIBG) increased cell killing in 35%–70% of recipient cells (cells not incubated with ^{123}I -MIBG), depending on the cell line used. The effect however, was only seen at low activity concentrations, while at higher activities, the bystander effect became progressively weaker (82). This effect was supported by results using ^{123}I -UdR (iodo-deoxyuridine), which is incorporated into the DNA during DNA synthesis, in a colon cancer cell line, which showed a dose-dependent decrease in the surviving fraction of bystander cells, but only at low concentrations. At higher concentrations the toxicity was relieved, suggesting that ^{123}I may elicit a toxic or protective effect on neighboring cells at low and high dose, respectively (83). In contrast, Kishikawa et al. found that ^{123}I induced stimulatory bystander effects in human colon cancer cells and increased survival both in vitro and in vivo, whereas ^{125}I -UdR showed toxic bystander effects (84). Inhibitory bystander effects were also observed with ^{125}I -UdR in vitro in chinese hamster V79 cells (85), in colon cancer cells (86) and in the breast cancer cell line MCF7 (84). However, in another breast cancer cell line (MDA-MB-231) no bystander effects were observed indicating that differences in the phenotypes of cells also could play a role in the induction of bystander effects (87). In vivo experiments using human colon carcinoma cells, labeled with a lethal dose of ^{125}I -UdR prior to mixing with unlabeled cells and before injection into nude mice showed a significant inhibitory effect on tumor growth due to bystander effects in the non-labeled cells (88). Understanding and mastering of bystander effects via smart nanomedical delivery systems with modulatory functionalities could represent one of the future routes.

Therapeutic aspects

To fully take advantage of AEs in cancer therapy, i.e., to exploit the “Auger effect”, it is imperative that the radio-nuclide is targeted to tumor cells, internalized, and transported to the nucleus in very close proximity to DNA, to which the electrons are damaging and lethal. A possible approach to improve the efficacy of AE therapy could be to amplify the potency of the targeted radiation by combining with other anti-cancer treatments e.g., radio-sensitizers, chemotherapy, small-molecule inhibitors, external irradiation or even gene therapy. Ultimately, this may warrant more sophisticated nanomedical delivery systems with, for example, timed release of different components.

The general feasibility of the conception has been demonstrated by some recent studies. Trastuzumab-resistant cell lines could be killed by ^{111}In and this effect was stronger when cells were radiosensitized by methotrexate, which further reduced clonogenic survival in breast cancer cells (28). But also other low-dose chemotherapeutics (paclitaxel and doxorubicin) sensitized breast cancer cells to ^{111}In -NLS-Trastuzumab, which was ascribed to changes in cell cycle distribution and/or changes in DNA damage repair by the chemotherapeutic drugs (89).

Cornelissen et al. used the combination of external radiation and the ^{111}In -anti- γH2Ax -tat conjugate in breast cancer cells and in xenografts. Cells were allowed to internalize the conjugate (via tat) and irradiated to allow the formation of γH2Ax , which is then targeted by the antibody bringing the AE into close proximity to the DNA. In vitro, there was an increase in γH2AX foci and a decrease in survival after external radiation combined with the radiolabeled conjugate. In vivo, the result was a decreased growth rate of the tumors compared to either treatment alone. Hence, the combination of external and internal therapy amplifies the DNA damage and increases the antitumor effect in vivo (46). This was further expanded, using a bi-specific conjugate, introducing EGF as part of the conjugate to allow tumor cell targeting, either in a cleavable or non-cleavable form, however only the cleavable form of the immune-conjugate accumulated in the nucleus after external irradiation and decreased clonogenic survival (90).

When EGFR activity was abolished by the small molecule inhibitor gefitinib, the nuclear accumulation of ^{111}In -EGF was significantly higher than without gefitinib and this was accompanied by an increase in the number of γH2Ax foci and reduced clonogenic survival in breast cancer cells (91).

The AE molecule ^{125}I -UdR was combined with gene therapy, by introducing the anti-tumor protein IFN γ into mouse hepatoma tumors in mice under the control of a radiation-sensitive promoter. This means that IFN γ is only expressed when the cells are irradiated, externally or internally. The tumors were injected with the IFN γ -DNA and ^{125}I -UdR in combination or alone. The combination decreased the tumor volume and the mean survival of the mice injected with both was significantly longer than with ^{125}I alone, whereas mice injected with IFN γ plasmid alone had the same survival as control mice (92).

Finally, when block copolymer micelles (BCMs) were conjugated with ^{111}In , an NLS sequence and a HER2 specific antibody and loaded with the radiosensitizer methotrexate, the micelles were taken up by HER2 overexpressing cells in vitro. ^{111}In accumulated in the nucleus and the concomitant low-dose methotrexate further reduced cell survival (93).

However, efficient delivery of therapeutic and imaging agents is hindered by macrophages which quickly detect and engulf nanoparticles. In the past, this has been circumvented by masking the nanoparticles with polyethylene glycol [reviewed in (94)]. A new promising approach has been the discovery of “self-peptides” i.e., a peptide sequence derived from cluster of differentiation 47 (CD47), which prevents phagocytosis by macrophages when attached to the nanoparticle surface (95). Additional self-peptides are likely to exist and could also be used to further avoid phagocytosis and thereby increase delivery of therapeutics and imaging agents to cancer cells.

Clinical use

^{111}In -octreotide was already tested for treatment of neuroendocrine tumors in patients in the late 1990s, and high doses led to symptomatic relief. However, tumor regression was rare with the most common long-term side effects being to the bone marrow (96–98). Several reasons may be responsible for these somewhat disappointing results, one being the choice of radionuclide. For these studies, ^{111}In was probably chosen for its availability, but it is a suboptimal therapeutic radionuclide providing a low so-called tumor-to-normal-tissue mean absorbed dose-ratio in comparison to much better suited AEs like ^{119}Sb or $^{58\text{m}}\text{Co}$ (99). The second reason is probably the lack of sufficient nuclear entry as this is a key precondition for a sufficient amount of DNA DSBs (72). Furthermore, for large, bulky tumors with heterogeneous uptake of the radiopharmaceutical, the lack of cross-fire into unlabeled cells from AEs may result in cells escaping the therapy. For such tumors a long-range β -emitter is better suited – or even better in combination with an AE in a “cocktail approach” to effectively kill the disseminated, circulating tumor cells and micro-metastases. This circumstance and a still limited but growing understanding of the biological effects of Auger processes may explain, why the significance of AEs has been largely ignored by the medical community despite their extreme localized radiotoxicity,

which in theory makes them ideally suited for targeted DNA damage in single cells and micro-metastases, and thus for treating of disseminated cancer with very limited side effects. A steadily increasing amount of knowledge on the characteristic and biological behavior of AEs has been collected since the possible benefits of their damaging effect were addressed by Feinendegen in 1975 (11). Despite the many promising observations and peculiar nature of the Auger processes, which in principle make them ideally suited for “internal” targeted radiation therapy as opposed to external irradiation, the clinical application is still in its infancy.

Perspectives

The future of AEs in medical use will no doubt be concentrated on cancer, since the vision is to target and kill all cancer cells. Due to the very short range of the emitted electrons it is generally accepted that AEs do not exhibit their extreme radiotoxicity, i.e., the “Auger effect”, unless situated very close to or in the DNA and, thus, they have unique properties not matched by other radioisotopes or external irradiation.

While AEs have great therapeutic potential in cancer therapy, the requirement of targeting not only all tumor cells but also their DNA presents a major hurdle. However, great progress has been made in recent years, albeit that many of the most promising approaches have so far only been tested in early preclinical settings and, hence, there is still room for improvement of the different phases of AE therapy.

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