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Molecular Methods in Nuclear Medicine Therapy

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Owing to the recent advancement in cancer biology and molecular biology techniques, the role of nuclear medicine (NM) as a therapeutic tool for cancer management is in rapid expansion. NM has traditionally contributed to molecular oncology by allowing noninvasive monitoring of tumor metabolism, growth and genetic changes, thereby providing a basis for appropriate biology-based treatment planning. However, NM techniques are now being applied as an active therapeutic tool in novel molecular approaches for cancer treatment. Such areas include research on cancer therapy with radiolabeled ligands or oligonucleotides, and utilization of synergism between NM radiotherapy and gene transfer techniques. Here we will focus on novel aspects of nuclear medicine therapy.

1. Advances in Targeted Radioligand Therapy Strategies

The prospects for radiolabeled ligands (such as MoAb or peptides) in cancer therapy are promising due to their specific binding to tumor antigens or receptors. One reason this approach has been less than successful is due to the relatively low tumor uptake of radioligands. This can be caused by a large distribution volume, poor tumor vascularization and permeability, heterogeneous and low expression of target molecules, or a short circulating half-life of the ligand. The following are methods that are being developed to increase tumor uptake of radio-labeled ligands in experimental studies.

1) Ligand Strategies for Increasing Tumor Localization

Ligands with greater affinity would increase radioligand uptake, retention, and their therapeutic efficacy. Ligands reactive with new tumor receptors or vascular endothelium which are highly accessible to systemically administered radioligands have been investigated. Indirect radioiodination techniques and labile bifunctional chelating agents have provided an improvement in tumor uptake and retention as well as antitumor effects.

Alterations in ligand glycosylation can change pharmacokinetics of body clearance, alterations in receptor binding regions can enhance binding affinity and specificity, whereas insertion of labeling sites may result in more efficient tumor

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cell killing with less toxicity to normal tissues.

Predosing with unlabeled MoAb before injection of a radiolabeled MoAb has resulted in higher tumor uptake and T/NT uptake ratios and improved therapeutic results in an animal model of lymphoma have been reported. Because of the heterogeneity of antigen expression, it has been proposed that a cocktail of MoAbs with different distributions within the tumor may lead to greater tumor localization and better therapeutic results. Multiple doses or continuous infusion of radiolabeled ligands may also result in improved tumor uptake and results as well as decreased hematologic toxicity, although altered tumor permeability and immune response remains an obstacle to multiple dosing.

2) Strategies for Altering the Blood Clearance of Radioligands

Reductions in ligand size will alter the pharmacokinetics of clearance, change metabolism patterns in the kidney or liver, and reduce immunogenicity. In general, antibody fragments and peptides have a more rapid catabolism which may lead to higher T/NT uptake ratios and lower radiation to bone marrow. In addition, antibody fragments and peptides penetrate tumors better and have a more homogeneous tumor distribution. Conversely, fragments and peptides clear from tumors faster and cause higher radiation to kidneys.

Methods are being developed to inhibit enzymatic destruction of peptides. Multimers of ligands can improve tumor localization as a result of higher affinity and slower blood clearance, and genetically engineered Ab fragments show potential for improvements in tumor targeting and therapy. Lysine infusion can block renal uptake of radiolabeled peptides and Fab fragments.

In pretargeting strategies, the radiolabel is administered on a carrier having a high binding affinity for a target molecule that was previously administered to allow sufficient time to localize in the target and clear from circulation and normal tissues. Biotin and avidin have assumed a dominant role in recent pretargeting studies. Pretargeting by DNA-DNA hybridization using 15-base single-stranded DNA has been demonstrated in vitro. Peptide nucleic acid, in which the DNA backbone has been replaced with a polyamide backbone, may also be used.

3) Tumor Strategies for Increasing Localization of Radioligands

Increasing antigen/receptor expression on tumor cells is an important method to enhance targeting. Interferon, for instance, increases expression of tumor-associated antigens resulting in increased tumor localization of radiolabeled MoA in clinical trials, but may also increase myelosuppression. Gene transfer techniques to increase antigen or receptor expression and tumor localization of radioligands are being actively investigated and is described in more detail below.

Tumor vascularity, blood flow, and vascular permeability are additional factors that affect the localization and distribution of radiolabeled ligands, as well as their therapeutic effect. Physiologic barriers to ligand movement in tumor include heterogeneous blood supply and elevated interstitial pressure in central tumor that opposes inward ligand diffusion. Approaches that modify these barriers include external irradiation, hyperthermia, and the use of vasoactive agents.

2. Peptide Receptor Imaging and Therapy

One of the most exciting aspects for the use of radiopeptides is their potential for therapy. The

somatostatin receptor is the most extensively studied system for this purpose. Radiolabeled analogues of octreotide are now in wide clinical use for imaging such tumors as meningiomas, paragangliomas, pheochromocytomas and lung cancer. Receptor positive tumors may also originate in pituitary adenomas, gastroentero-pancreatic tumors, pancreatic islet cell tumors, lymphmas, and breast cancer.

Because of a long biologic half life for ^{111}In in tumor tissue, ^{111}In labeled [DTPA^{0}]octreotide has an appropriate distribution profile for radionuclide therapy. In a clinical trial on end stage neuroendocrine tumor patients with a typical dose per administration of 6-7 GBq, the critical organs were the kidneys and spleen. Radiation does to was 17-67 Gy for tumors of 10 gm size, and tumor reduction was observed in 6 of 21 patients. In order to overcome the small particle range of Auger electrons from ^{111}In, work is in progress to label octreotide with α or β emitting isotopes. A recent phase I trial in 22 end stage cancer patients using ^{90}Y-[DOTA^{0}, Tyr^{3}]octreotide demonstrated a partial response in 2, minor response in 3, and stable disease in 10 patients. A phase II trial on SCLC and breast cancer patients is expected soon.

Other potential therapeutic receptor targeting radiopeptides include cholecystokinin-B/gastrin receptor ligands or epidermal growth factors labeled with such isotopes as ^{111}In, ^{131}I, or ^{90}Y. As radiolabeled peptides are hoped to be the next magic bullet, many more novel radiopeptides for cancer therapy are currently under investigation.

3. Enhanced NM Therapy through Gene Modulation

There is presently intensive research in the treatment of cancer using selective transfer of therapeutic genes. Although still in the early stages of development, such approaches are recently showing promising results. For the NM field, gene therapy may also be utilized to enhance radioligand targeting to tumor cells. While the utilization of the sodium/iodide symporter gene is best known, numerous other genes that can be used to augment specific radioligand accumulation are also being investigated.

1) Sodium/Iodide Symporter Radio-tracer Concentrator Gene Therapy

The characteristic feature of differentiated thyroid carcinoma cells of accumulating iodide enables successful treatment with radioiodide. Iodide trapping in thyroid tissue is initiated by the active transport of iodide by NaI symporter (NIS) gene products on the cell membrane, and reduced iodide uptake in some thyroid cancers are attributed to a decreased expression of the gene. In an attempt to utilize the NaI symporter system for efficiently killing of tumors with radioiodide, work is being done with cancer gene transfer with the NaI symporter gene.

Mandell et al. (1999 Cancer Res) utilizied a retroviral vector containing the NIS gene under SV40 promoter control to transduce human cancer cell lines and found augmented iodine uptake as well as enhanced cell killing in vitro. In addition, NIS gene transduced xenografts in mice were well visualized in vivo after I-123 injection. Haberkorn et al. (2001, J Nucl Med) were able to induce sufficient uptake of iodine in hepatoma cells after NIS gene transduction, and observed a 6 fold higher iodine uptake in transduced tumors in vivo.

2) Cell Receptor Radiotracer Concentrator Gene Therapy

Growth factors (GF) play a vital role in

intracellular signaling and exert their effects by binding to their receptors. GF receptor mediated targeting strategies are very attractive for cancer applications because many of the identified cellular oncogenes encode either GFs or their receptors which offer opportunities for enhanced radioligand targeting to tumors.

Raben et al (1996, Gene therapy) first tested the approach by enhancing tumor uptake of I-131 anti-CEA MoAb in glioma xenografts after transfer of the CEA gene. Rogers et al (1997, Cancer) transfected ovarian tumors in mice with adenovirus containing the GRPr gene and found a 30 fold increase in tumoral uptake of a radiolabeled Bombesin analogue. Radiolabeled anti-erbB-2 MoAb targeting of intraperitoneal tumor xenografts after gene transfer of the erbB gene has also been investigated.

The somatostatin system has been the recent focus as a therapeutic target. Zinn et al. (J2000, J Nucl Med) observed a 5-10 fold increase in radiolabeled somatostatin anologue in vivo uptake in lung cancer xenografts after injecting adenovirus containing the SSTr2 gene. Rogers et al. (1999, Clin Cancer Res) demonstrated the effect of adenoviral SSTr2 gene transfer in lung cancer cells on In-111 labeled octreotide. Other potential tragets may include platelet derived GFR, epidermal GFR, fibroblast GFR, Insulin-like GFR, neurotrophin R, CCK R, vasoactive intestinal peptide (VIP) R, and substance-P receptors.

4. Radiation Enhanced Gene Therapy

In addition to radiotracer concentrator gene therapy, there are a variety of other therapeutic strategies that combine gene therapy with NM therapy for cancer. These include the use of vectors that contain a radiation-inducible promoter, and vectors that express proteins to enhance radiosensitivity. NM imaging can also be used to monitor efficiency of gene transfer and transgene expression during gene therapy, and NM therapy may augment gene transfer efficiency by local radiation effects. Finally, there is a rising interest in the synergistic effects between radiation therapy and other molecular or gene therapy approaches.

1) Use of Radiation to Modulate Transgene Expression

One approach to improve the temporal and spacial specificity of gene therapy involves using radiation-inducible promoters to activate gene expression selectively by ionizing radiation. The expression of some early response gene is up-regulated by exposure to ionizing radiation. These genes include those encoding the human tPA, TNF-α, and Egr-1(early growth response protein-1).

The radiation inducibility of the Egr1 promoter has been exploited in several experimental gene therapy strategies. The combination of adenoviral gene transfer of the tumoricidal TNF-α controled by the Egr-1 promoter and 50Gy radiation to human tumor xenografts resulted in increased tumour control compared with treatment with Ad5.Egr-TNF or radiation alone (1995 Nat Med). In another experiment, irradiation of intracerebral tumors infected with the adenovirus containing the lacZ gene driven by the Egr-1 promoter using [^{125}I]IdUrd resulted in increased β-galactosidase reporter activity of glioma cells (1997, Gene Ther). Other promoters are also being investigated such as the WAF1 promoter which can induce a 5-10 fold increase of transgene protein expression after 4Gy of radiation (2000, Gene Ther).

2) Radiation Enhanced Gene Transfer

While the vast majority of present gene therapy

protocols rely on viral vectors or plasmid vectors for gene transduction, there is continued effort to increase the efficiency of gene transfer into target cells, leading to sufficient expression of the therapeutic protein. It has recently been observed that ionizing radiation can improve both plasmid and viral gene transfection efficiency in normal and neoplastic cells (1996, Hum Gene Ther). For plasmid DNA, as high as 1,400-fold improvement in transfection efficiency was seen using 9 Gy radiation in human fibroblasts, and the effect was dose-dependent. Radiation has been shown to greatly improve transfection efficiency of adenoviral vectors also, and resulted in adenoviral genome integration into cellular DNA, which should prolong transgene expression (1997, Hum Gene Ther).

Thus, pretreatment of cells with ionizing radiation appears to improve both immediate transduction efficiency and duration of transgene expression. Such findings may lead to the development of future protocols combining radiation and gene therapy in treating human malignancy.

3) Synergistic Tumoricidal Effects between Radiation and Molecular Therapy

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