Pivotal role of nanotechnologies and biotechnologies for molecular imaging and therapy

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MicroSPECT and microPET are increasingly used in oncology, neurology and cardiology to analyse molecular alterations in vivo in animal models of diseases. In particular, microPET is the principal in vivo molecular imaging technique, and is already extensively used to assess numerous variables related to physiological and pathological states in the mouse. Other methods that are based on different physical principles, such as magnetic resonance imaging and spectroscopy and bioluminescence techniques, are also used for molecular imaging, for research on animal models.

These tremendous developments in instrumentation technology are paralleled by developments in radiochemistry, radiochemical technology and biotechnology, with continuous attention being paid to micro- and nanotechnology. In particular, over the past few months, new technological approaches to the synthesis of radiotracers have been proposed by a few research teams while new genetically engineered mice and cellular clones, along with labelling techniques of different reporter products, have also been described. These new technologies are improving day by day the applications of the imaging methodologies and our ability to understand diseases, trace cellular and molecular processes in vivo, and monitor the efficacy of treatments performed with the most advanced techniques, including the use of alpha-emitting radionuclides and stem cells.

The commentaries in this section derive from a literature search and include summaries of articles compiled and linked to each other by extensive use of the text contained in the articles examined.

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Micro-reactor nanotechnology for radiochemistry

Continuous-flow micro-reactors have been used for chemical processes on the nanolitre to microlitre scale. Micro-reactor devices consist of a network of micron-sized channels (typical dimensions in the range 10–300 μm) embedded in a solid substrate. These devices have very small dimensions, 10 mm in diameter and 0.1 mm in depth, and permit the manipulation and transfer of very small quantities of fluid to achieve a chemical synthesis, within an integrated circuit. Miniaturisation of radio-syntheses might lead to the use of smaller quantities of expensive precursors than are presently required, and to easier purification processes with greater yield and specific activity. Micro-reactors are now emerging as an extremely useful technology for the intensification and miniaturisation of chemical processes.

Synthesis of [^{18}F]FDG in an integrated microfluidic device

Chung-Cheng Lee and colleagues, from a broad range of institutions in California, have reported the synthesis of ^{18}F-fluorodeoxyglucose ([^{18}F]FDG) in an integrated microfluidic device [1]. Five sequential processes—[^{18}F] fluoride concentration, water evaporation, radio-fluorination, solvent exchange and hydrolytic deprotection—resulted in a high radiochemical yield and purity and entailed a shorter synthesis time relative to conventional automated synthesis. Multiple doses of [^{18}F]FDG for positron emission tomography imaging studies in mice were prepared. They also designed a chemical reaction circuit with the capacity to synthesise large [^{18}F]FDG doses. The chip has a coin-shaped reactor (volume 5 ml) equipped with a vacuum vent. It was used to synthesise 1.74 mCi of [^{18}F]FDG, an amount sufficient for several mouse experiments. From the purified and sterilised product, two doses (375 mCi and 272 mCi) were used for microPET molecular imaging of two mouse models of cancer. The authors concluded that their results, which constitute a proof of principle for automated multistep

syntheses at the nanogram to microgram scale, may be generalised to a range of radiolabelled substrates.

Radio-halogenation of small and large molecular weight molecules with a microfluidic device

Gillies et al., in two distinct yet very similar papers [2, 3], have reported the radio-halogenation of small and large molecular weight molecules using the microfluidic device. These reactions involved the direct radio-iodination of the apoptosis marker annexin V using ^{124}I, the indirect radio-iodination of the anti-cancer drug doxorubicin from a tin-butyl precursor and the radiosynthesis of [^{18}F]FDG from a mannose triflate precursor and ^{18}F. They demonstrated the rapid radio-iodination of the protein annexin V (40% radiochemical yield within 1 min) and the rapid radio-fluorination of [^{18}F]FDG (60% radiochemical yield within 4 s) using a polymer microreactor chip. Chromatographic analysis showed that the labelling efficiency of the unoptimised microfluidic chip is comparable to that of conventional PET radiolabelling reactions.

Hydrodynamically driven micro-reactor to label carboxylic esters with positron emitters

It should be mentioned that the use of a hydrodynamically driven micro-reactor had previously been proposed by Shui-Yu Lu [4] from the National Institutes of Health in Bethesda, Maryland, USA, to label carboxylic esters with one of two short-lived positron emitters, ^{11}C or ^{18}F. The authors proved the feasibility of various syntheses with different radiochemical yields depending on the infusion rate. Their results exemplify the advantages of the micro-reactor methodology for production of radiotracers, which include the use of small quantities of substrates, rapid reaction optimisation and easy product purification.

Molecular and cellular biotechnologies for imaging and therapy development

Three recently published papers highlight the synergistic potential of the rapidly evolving areas of molecular and cellular biotechnology, animal engineering and radionuclide-based imaging and therapy.

Affibodies: small radiolabelled targeting proteins for visualisation of tumours in vivo

Anna Orlova et al. [5], from the Affibody AB, Bromma and Department of Oncology, Radiology, and Clinical Immunology, Rudbeck Laboratory, Uppsala University, Sweden, focussed on the detection of cell-bound proteins that are produced due to aberrant gene expression in malignant tumours and that can provide important diagnostic information influencing patient management. They theorised that use of small radiolabelled targeting proteins would enable high-contrast radionuclide imaging of cancers expressing such antigens if adequate binding affinity and specificity could be provided. In their paper they describe a HER2-specific 6-kDa Affibody molecule with 22 pmol/l affinity that can be used for the visualisation of HER2 expression in tumours in vivo using a gamma camera. They constructed a library for affinity maturation by re-randomisation of relevant positions identified after the alignment of first-generation variants of nanomolar affinity (50 nmol/l). One selected Affibody molecule, ZHER2:342, showed a >2,200-fold increase in affinity achieved through a single-library affinity maturation step. When radio-iodinated, the affinity-matured Affibody molecule showed clear, high-contrast visualisation of HER2-expressing xenografts in mice as early as 6 h post injection. The tumour uptake at 4 h post injection was improved fourfold (due to increased affinity), with 9% of the injected dose per gram of tissue in the tumour. The authors concluded that Affibody molecules represent a new class of affinity molecules that can provide small-sized, high-affinity cancer-specific ligands which may be well suited for tumour imaging, and that if the molecule were to be tested in the clinic, ^{123}I or ^{124}I could be used. Furthermore, they observed that the development of suitable chemistry to label the ZHER2:342 molecule with generator-produced ^{99m}Tc or ^{68}Ga could further facilitate future imaging studies.

^{211}At therapy against a thyroid carcinoma cell line genetically modified to express NIS (K1-NIS)

In another paper, Petrich et al. [6], from the Klinik für Nuklearmedizin, Medizinische Hochschule Hannover, Germany and the Institut für Pathologie, GSF-Forschungszentrum für Umwelt und Gesundheit, Neuherberg, Germany, have reported on the in vivo effects of the high linear energy transfer (LET) emitter radioastatine (^{211}At) on tumour growth and outcome in nude mice. They carried out their study based on the fact that sodium/iodide symporter (*NIS*) gene is currently being explored in several trials to eradicate experimental cancer with ^{131}I by its beta-emission. The same authors have recently characterised NIS-specific cellular uptake of an alternative halide, ^{211}At, which emits high-energy alpha-particles. In the present study they administered ^{211}At in a fractionated therapy scheme to NMRI nude mice harbouring rapidly growing solid tumours established from a papillary thyroid carcinoma cell line genetically modified to express NIS (K1-NIS). Animals were observed over 1 year. Tumour growth, body weight, blood counts, survival and side-effects were measured in comparison with control groups without therapy and/or lack of NIS expression. It was observed that within 3 months, ^{211}At caused complete primary tumour eradication in all cases of K1-NIS tumour-bearing nude mice (n=25), with no tumour recurrence during 1 year of follow-up. Survival rates of the K1-NIS/^{211}At group were 96% after 6 months and 60% after 1 year, in contrast to those of control

groups (maximum survival 40 days). The authors concluded that ^{211}At represents a promising substrate for NIS-mediated therapy of various cancers with either endogenous or gene transfer-mediated NIS expression.

In vivo visualisation of stem cell survival, proliferation and migration after cardiac delivery

Finally, in a third paper Cao et al. [7], from the Department of Radiology, Bio-X Program, Stanford University School of Medicine, California, USA, have reported on the in vivo visualisation of embryonic stem cell survival, proliferation and migration after cardiac delivery. Their study was based on recent findings showing that stem cell therapy can promote tissue regeneration. Monitoring stem cells in vivo is a major challenge owing to the limitations of conventional histological assays and imaging modalities. The authors stably transduced murine embryonic stem (ES) cells with a lentiviral vector carrying a novel triple-fusion (TF) reporter gene that consists of firefly luciferase, monomeric red fluorescence protein and truncated thymidine kinase (fluc-mrfp-ttk). ES cell viability, proliferation and differentiation ability were not adversely affected by either reporter genes or reporter probes compared with non-transduced control cells. Afterwards, ES cells carrying the TF reporter gene (ES-TF) were injected into the myocardium of adult nude rats. Control animals received non-transduced ES cells. At day 4, the bioluminescence and PET signals in study animals were significantly higher than in controls. Both signals increased progressively from week 1 to week 4, which indicated ES cell survival and proliferation in the host. Histological analysis demonstrated the formation of intracardiac and extracardiac teratomas. Finally, animals (n=4) that were treated with intraperitoneal injection of ganciclovir (50 mg/kg) did not develop teratomas when compared with control animals (n=4) treated with saline (1 ml/kg). The authors concluded that this is the first study to characterise ES cells that stably express fluorescence, bioluminescence and positron emission tomography reporter genes and monitor the kinetics of ES cell survival, proliferation and migration. They also suggested that this versatile imaging platform should have broad applications for basic research and clinical studies on stem cell therapy. It must be noted, however, that the authors also raised a concern: that of stem cell misbehaviour. The possibility of teratoma formation after transplantation poses a daunting challenge for clinical application of ES cells. To date, no study has addressed this issue from an imaging standpoint. The authors hypothesised that a PET reporter gene (*ttk*) could also serve as a suicide gene using ganciclovir treatment.

Conclusions

There is no shortage of challenges and opportunities for molecular imaging nowadays. The growth of nanotechnologies applied to radiochemical syntheses, besides guaranteeing reproducible techniques, opens the way to simpler and more cost-effective procedures for molecular imaging. This will result in wider use, appreciation and success of both. Using microfluidic reactions the yields of radiopharmaceutical syntheses using short and medium half-life radionuclides can be further improved by optimisation of the microfluidic devices and of fluid mixing profiles.

What emerges from examples of the integration of nano- and biotechnology into molecular imaging and therapy studies is a vision of the future, a representation of great opportunities for molecular medicine to exploit the potential of imaging to develop, pursue and monitor the science of individualised treatment.

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