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Y imager

Application Notes

Improved apoptosis imaging in tumor bearing mice by ^{99m}Tc -AFIM planar scintigraphy

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Goal:

The objective of the present study is to assess the biodistribution of a new ^{99m}Tc -labeled radiotracer in order to detect and quantify apoptosis in tumors following chemotherapy. An early event in apoptosis is redistribution of phosphatidylserine (PS) from the inner or outer leaflet of the cell membrane. We have designed a 9 kDa miniprotein, AFIM, engineered from the annexins' domains topology and knowledge of specific PS binding sites. AFIM has a high thermodynamic stability, reversible folding, and a strong affinity for PS-containing membranes. We have engineered a multipurpose site both on AFIM and Annexin 5 that can be used either for labeling with ^{99m}Tc or for coupling to another imaging agent.

Materials and methods:



All imaging was performed with the γ IMAGER (Biospace Lab, Paris), a planar scintigraphic camera that combines a continuous 4 mm thick x 120 mm diameter CsI(Na) crystal with a 5 inch dia. position-sensitive Hamamatsu R3292 photomultiplier. The result is a high-resolution camera with a circular 100 mm dia. field of view.

While other high-resolution cameras employ pixellated crystals, in the γ IMAGER spatial resolution is calculated with a look-up table that associates the actual position with the coordinates determined by the photomultiplier as each event is detected. Pulse height of the detected event is associated to energy according to a spatially dependent energy correction table.

For ^{99m}Tc , the intrinsic spatial and energy resolutions are 2 mm and 11% respectively.

The γ IMAGER is available with various parallel-hole collimators for optimized performance with different isotopes, as well for resolution/speed tradeoffs. In the present study, the γ IMAGER was equipped with a parallel-hole collimator 35 mm high, having 1.7 mm dia. holes and 0.2 mm septa.

In a first step:

The biodistribution of AFIM was measured on control mice. Control mice (T57 Black6) were injected I.V. with 100 μCi ^{99m}Tc -AFIM and anesthetized through Forene breathing nosecones over a 6-hour period. The biodistribution was measured by planar scintigraphy via the γ IMAGER in 5-minute segments over the entire 6 hours. A direct comparison of biodistribution was made under the same conditions with mice dosed with ^{99m}Tc -Annexin 5, a common apoptosis tracer. Both distributions were simultaneously imaged with the mice on their breathing platforms set directly on the collimator.

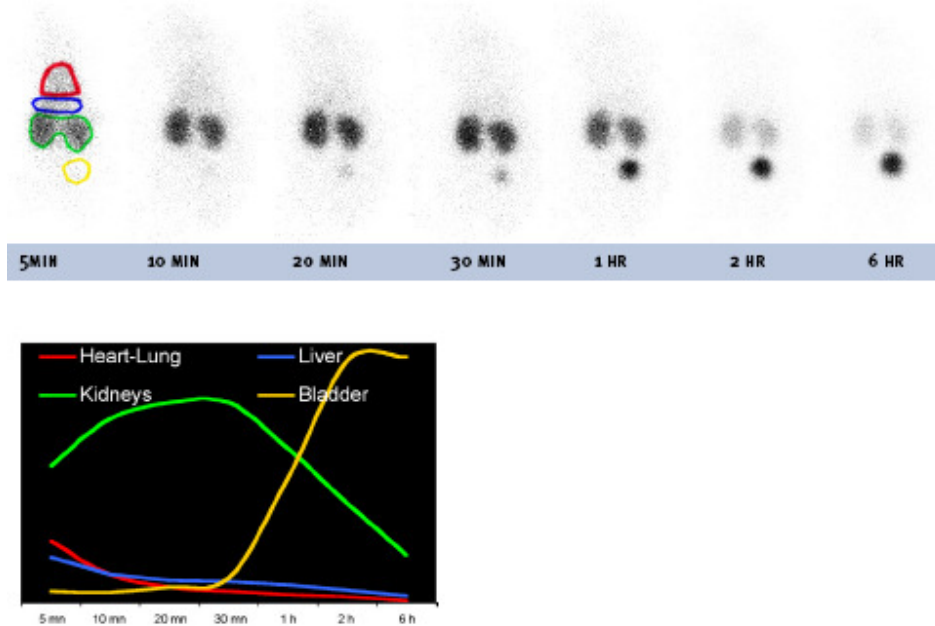
In a second step:

The specificity of ^{99m}Tc -AFIM and its biodistribution on diffuse melanoma was assessed. ^{99m}Tc -AFIM and ^{99m}Tc -Annexin 5 were used in several mouse models of tumors undergoing chemotherapy to induce apoptosis in a diffuse melanoma model in Ret+/- mice and in melanoma tumors transplanted in Ret+/- mice.

Mice were administered chemotherapy (Cisplatin 30 mg - Navelbin 60 mg) at day 1, 8, 15 and 21. Planar scintigraphies were performed prior to chemotherapy and at 24, 48 and 72 hours thereafter. All scintigraphies were performed one hour after 300-400 μCi of ^{99m}Tc -AFIM was administered I.V. Images were acquired in 30-minute exposure times. Tumor apoptosis was controlled post mortem by histochemistry using the TUNEL assay (Promega).

Results:

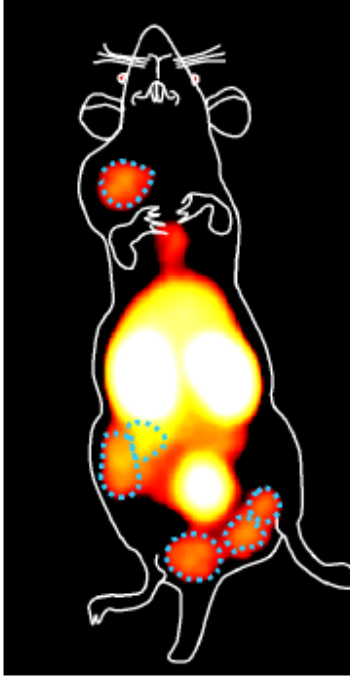
The biodistribution of ^{99m}Tc -AFIM is shown. Using the Biospace Lab γ VISION + software, automatic contouring and tracing of region of interest activity resulted in the curves displayed below. Clearly there is fast renal clearance of ^{99m}Tc -AFIM, while part of the ^{99m}Tc -Annexin 5 clearance follows the hepato-biliary route.



Tumor bearing mice that had undergone successful chemotherapy showed identifiable regions of interest from apoptotic tumors as early as 24 hours after the first injection.

An example of apoptotic tumor (melanoma) imaging obtained 72 hours after a fourth injection of 100 μg Navelbin (see left) shows tumors with activity ranging from three to six fold that of body background. Correlation of the quantitation performed after imaging and post mortem assay measurements of apoptotic cell concentration is currently under investigation.

Conclusion:



The use of the γ IMAGER has allowed us to quickly assess the biodistribution and specificity of ^{99m}Tc -AFIM on a number of tumor models. Preliminary results of this study are:

- ^{99m}Tc -AFIM is excreted only through the kidney. Blood kinetics of ^{99m}Tc -AFIM show its rapid disappearance within 30 min. No uptake of ^{99m}Tc AFIM is found in other organs apart from the kidney. Liver is slightly labeled. On the other hand, ^{99m}Tc -Annexin 5 is excreted partly through the kidney but mainly via the hepato-biliary route.
- Apoptosis can be detected as early as 24 hours after successful chemotherapy and is clearly observed after 72 hours with either AFIM or Annexin 5. ^{99m}Tc -AFIM has been shown to recognize apoptotic cells in several tumor models.

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