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Title: Flexible ^{64}Cu-nanoparticle-based cell labeling system allows for *in vivo* tracking of adoptively transferred T-cells by PET/CT

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Background

To fully exploit the potential of adoptive cell transfer (ACT) therapy, an improved understanding of in vivo T-cell trafficking and engraftment following transfer is paramount. In vivo tracking of adoptively transferred cells by PET/CT represents an attractive imaging technology for ACT due to the quantitative capacity with high spatial and temporal resolution [1]. We have developed a flexible ^{64}Cu-micelle-based cell labeling system, which allows for prolonged cell tracking.

Methods

The labeling protocol was optimized for T-cell ^{64}Cu-micelle labeling in vitro and >90% loading efficiency was achieved with intact cell viability. Stability of the ^{64}Cu-micelles labeled T-cells was evaluated by co-incubating the T-cells with celltrace stained splenocytes for three and 24 hours. Following co-incubation, cells were FACS-sorted and ^{64}Cu activity in population determined. The radiolabeled T-cell population displayed a 10-14-fold higher ^{64}Cu activity level compared to celltrace^{+} splenocytes. In vivo, labeled T cell biodistribution and tumor accumulation in mice were investigated using PET/CT imaging in relation to radiation and various cancer immunotherapies. PET/CT scans were performed at multiple time-points up to 40 hours after ACT.

Results

Whole-body irradiated mice displayed significantly higher ^{64}Cu activity (4.6$\pm$0.1% of injected dose) in the thymus compared to controls (2.1$\pm$0.1% of injected dose). Additionally, there was correspondence between T cell number of the injected clone in

selected organs and radioactivity. This demonstrates that the signal is T-cell specific and retained in vivo in T-cells over time. We investigated the activity of labeled T-cells in tumors treated with an intratumoral sustained release depot of a TLR7 agonist (TLR7 Immunogel). The TLR7 Immunogel has been demonstrated to increase tumor protein levels of chemokines; CXCL-10, IFN-γ, and TNF-α, with importance for T-cell accumulation. PET/CT imaging showed that accumulation of ACT labeled T-cells was increased by pre-treating tumors with TLR7 Immunogel.

Conclusions

This flexible radiolabeling technology provides a novel method for tracking T-cells in vivo over several days. The flexibility of the system offers labeling of multiple murine and human cell populations, for high-resolution in vivo cell tracking.

Ethics approval

The study has been approved by the The Danish Animal Experiments Inspectorate.

References

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