Immunocompromised Human Serum Albumin/Human Neonatal Fc Receptor Mouse Model: A New Xenograft Model for Efficacy Studies of Immunotherapies

07/01/2022

One of the primary challenges scientists face in immunotherapy is to find an animal model that not only recapitulates the elements and characteristics of the human cancer they are studying, but also accurately predicts the efficacy, safety and toxicity of the potential drug candidate they are trying to develop.[1] Immunocompetent and immunocompromised mice grafted with tumor cell lines or tumors from patients represent valuable tools for such studies, as they allow the investigation of cancer physiopathology, and delineation of the efficacy and toxicity of immunotherapeutics.[2,3]

Another problem scientists confront when working in oncopharmacology is selecting the best formulation of their candidate drug, i.e., the one that targets precise regions of the body while maximizing the quantity and duration of its exposure. Indeed, since therapeutics are often low-molecular-weight molecules, they have rapid renal clearance, short plasma circulatory time, and nonspecific distribution, all contributing to a weak, short and diffuse therapeutic action.[4] To overcome these obstacles, tremendous efforts have been made to generate drug delivery systems, i.e., engineered technologies for the targeted delivery and/or controlled release of therapeutic agents. One such technology is the human serum albumin (HSA), the most abundant protein in the blood (60% by mass) that, over the past decades, has become a pivotal contender as a molecular cargo and nano vehicle in biophysical, clinical and industrial fields, due to its extraordinary capacity to bind, store and transport a wide variety of endogenous and exogenous ligands, including pharmacological drugs.[5]

Developed by genOway in collaboration with Albumedix, the first mouse model to express HSA and the human neonatal Fc receptor (hFcRn) was published in 2016 by a group of researchers, led by Kenneth A. Howard in 2016.[6] Compared to the historical Tg32 and Tg276 models, both HSA and hFcRn are controlled by their respective endogenous promoters in the HSA/hFcRn mouse, and the expression of both murine SA and FcRn is abolished, thereby avoiding cross-species differences in the albumin/FcRn interaction.[6–8] Thanks to these characteristics, the HSA/hFcRn model has been used successfully to perform preclinical studies of albumin-based drugs, conventional drugs, and biologics whose action is influenced by reversible binding to endogenous HSA.[6]

A few years later, the very same group turned to genOway again to develop a new, upgraded HSA/hFcRn model invalidated for Rag1. The resulting HSA/hFcRn/Rag1$^{-/-}$ mice, referred to as the AlbuMus RAG1 KO model, are immunocompromised, as they display deficiencies in T- and B-cell development (Figure 1).[9]

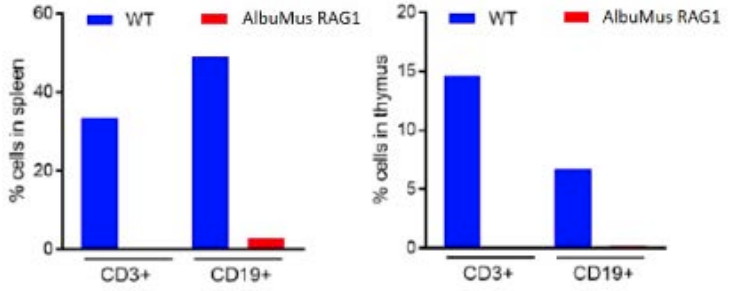

Figure 1 | Flow cytometry analysis of CD3⁺ T-cells and CD19⁺ B-cells in AlbuMus RAG1 KO mice. Freshly isolated splenocytes (left) and thymocytes (right) from AlbuMus RAG1 KO mice were analyzed and compared to wild-type mice (WT).

Adapted from Mandrup et al., Communications Biology, 2021.

The authors also showed that these mice can successfully grow human tumors when inoculated with human cancer cell lines.[9] The model was developed to study the anti-tumor efficacy and pharmacodynamic profile of a bispecific light T-cell engager (anti-EGFR nanobody x anti-CD3 scFv; LiTE) antibody genetically fused to HSA variants engineered with either null, wild type or high binding (HB) human FcRn affinity.

The results of this study, published in 2021 in Communications Biology, reveal that HSA/hFcRn/Rag1$^{-/-}$ mice subcutaneously inoculated with the human HT-29 colorectal cancer cell line are susceptible to tumor growth, similarly to C57BL/6 Rag1$^{-/-}$ animals (Figure 2A). Moreover, the authors found that mice injected with HSA–LiTE-HB fusion display greater tumor growth retardation compared to those treated with cetuximab (a commercial anti-EGFR antibody) or the other LiTE fusions (Figure 2B), suggesting that the HSA/hFcRn/Rag1$^{-/-}$ model allows accurate anti-tumor investigations of an anti-EGFR x anti-CD3 bispecific HSA fusion in tumors nonresponsive to standard anti-EGFR monoclonal therapies. Finally, analysis of serum levels showed that the albumin fusion Albu-LiTE-HB has an increased half-life compared to LiTE (Figure 2C).[9]

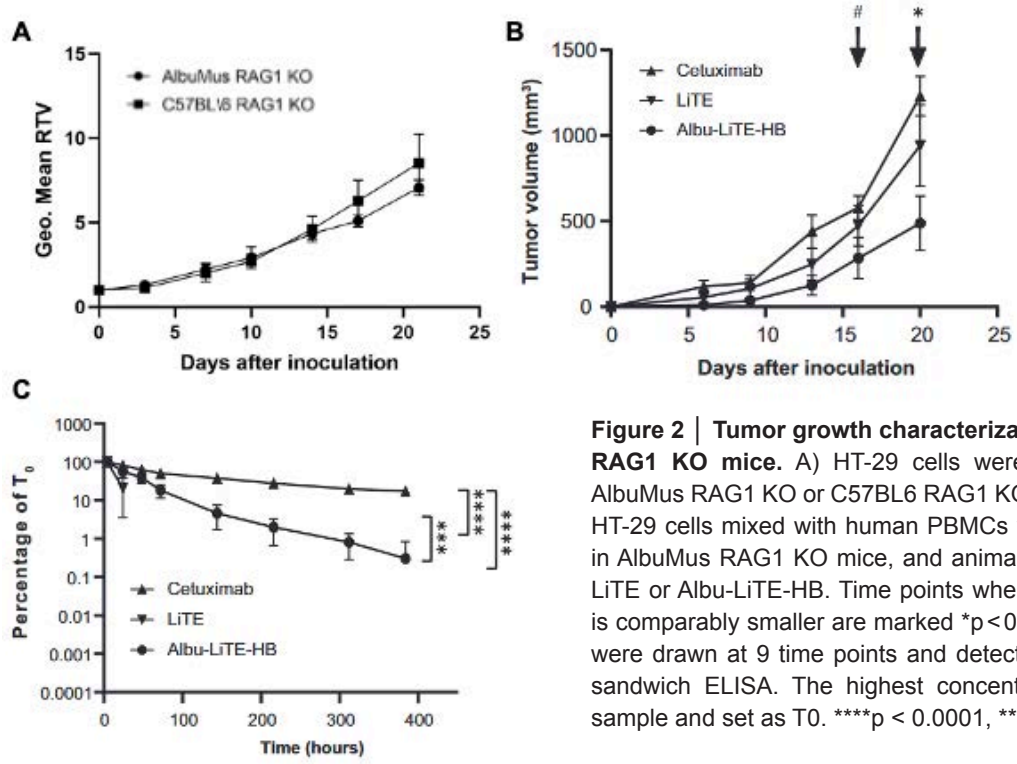

Figure 2 | Tumor growth characterization and inhibition in AlbuMus RAG1 KO mice. A) HT-29 cells were inoculated subcutaneously in AlbuMus RAG1 KO or C57BL6 RAG1 KO mice to follow tumor growth. B) HT-29 cells mixed with human PBMCs were inoculated subcutaneously in AlbuMus RAG1 KO mice, and animals were injected with cetuximab, LiTE or Albu-LiTE-HB. Time points where the Albu-Lite-HB tumor group is comparably smaller are marked *p < 0.01, #p < 0.05. C) Blood samples were drawn at 9 time points and detection in serum was performed by sandwich ELISA. The highest concentration was seen in the 4-hour sample and set as T0. ****p < 0.0001, ***p = 0.001.

Adapted from Mandrup et al., *Communications Biology*, 2021.

Importantly, human peripheral blood mononuclear cells (PBMCs) need to be co-injected with the tumor cell line and tested compound, thus providing an unusual distribution and infiltration of immune cells in the tumor micro environment. This specific point was discussed by Kenneth A. Howard during the Q&A session of his talk at genOway's first Bespoke Event.

These data show that the HSA/hFcRn/Rag1$^{-/-}$ model represents a valid tool for xenograft studies, and to assess the therapeutic efficacy and pharmacokinetics of drugs in primary immunodeficiency diseases, immuno-oncology and infectious diseases.

References:

1. Ireson, C. R., Alavijeh, M. S., Palmer, A. M., Fowler, E. R. & Jones, H. J. The role of mouse tumour models in the discovery and development of anticancer drugs. *Br J Cancer* 121, 101–108 (2019).
2. Capasso, A. et al. Characterization of immune responses to anti-PD-1 mono and combination immunotherapy in hematopoietic humanized mice implanted with tumor xenografts. *j. immunotherapy cancer* 7, 37 (2019).
3. Tentler, J. J. et al. RX-5902, a novel β-catenin modulator, potentiates the efficacy of immune checkpoint inhibitors in preclinical models of triple-negative breast Cancer. *BMC Cancer* 20, 1063 (2020).
4. Steinmetz, K. L. & Spack, E. G. The basics of preclinical drug development for neurodegenerative disease indications. *BMC Neurol* 9, S2 (2009).
5. Al-Harthi, S., Lachowicz, J. I., Nowakowski, M. E., Jaremko, M. & Jaremko, Ł. Towards the functional high-resolution coordination chemistry of blood plasma human serum albumin. *Journal of Inorganic Biochemistry* 198, 110716 (2019).
6. Viuff, D. et al. Generation of a double transgenic humanized neonatal Fc receptor (FcRn)/albumin mouse to study the pharmacokinetics of albumin-linked drugs. *Journal of Controlled Release* 223, 22–30 (2016).
7. Terje Andersen, J., Bekele Daba, M., Berntzen, G., Michaelsen, T. E. & Sandlie, I. Cross-species Binding Analyses of Mouse and Human Neonatal Fc Receptor Show Dramatic Differences in Immunoglobulin G and Albumin Binding. *J. Biol. Chem*. 285, 4826–4836 (2010).
8. Proetzel, G. & Roopenian, D. C. Humanized FcRn mouse models for evaluating pharmacokinetics of human IgG antibodies. *Methods* 65, 148–153 (2014).
9. Mandrup, O. A. et al. Programmable half-life and anti-tumour effects of bispecific T-cell engager-albumin fusions with tuned FcRn affinity. *Commun Biol* 4, 310 (2021).