Establishment of a reliable metastasizing syngeneic breast cancer mouse model using orthotopically implanted 4T1 cells after several rounds of isolating and reimplanting lung metastases

Andreas Lingnau, Steffen Hoffmann, Sandra Moor, Cynthia Schaefer-Obodozie, and Christoph Schaechtele

Reaction Biology Europe GmbH

Introduction

Many human breast cancer cell lines have been implanted into immunodeficient mice in order to establish in vivo xenograft models, subcutaneous or orthotopic variants, mimicing this type of cancer as good as possible. However, especially the metastasizing capabilities of these models turned out to be highly disappointing. In order to get more reliable models, breast cancer cells have often been implanted intravenously or intracardially rather than orthotopically. These models do work fine but resemble only limited parts of the complicated metastasis process. Therefore, a metastasis event originated from an orthotopically growing breast tumor seems more appropriate and displays an expanded view on the process of metastasis.

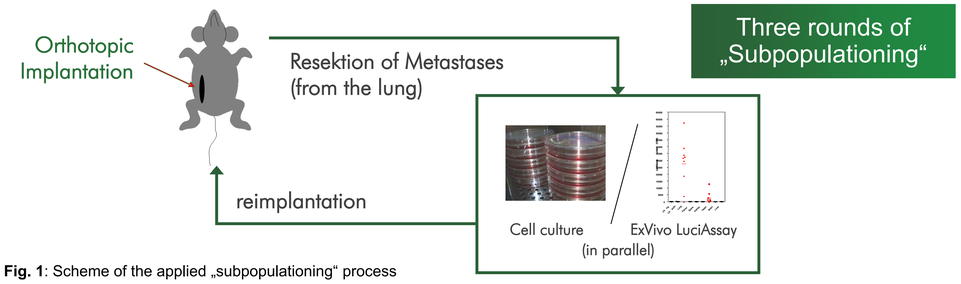


Fig. 1: Scheme of the applied „subpopulationing“ process

“Subpopulationing” as a tool to generate more reliable orthotopic Metastasis Models

The murine breast cancer cell line 4T1 was transduced with fire fly luciferase and implanted orthotopically into the fat pad of female BALB/C mice. In order to get higher metastasis rates we improved this model by resecting and recultivating the metastases of the invaded lungs after orthotopic implantation of the 4T1 cells.

Cultured cells were checked for in vitro luciferase activity in parallel with ex vivo analysis of the lung tissue part which was used for the isolation of the cells. After using this approach for several rounds, what we refer to as “subpopulationing” (Fig. 1), we checked for in-creased metastasis properties of this newly isolated breast cancer cells.

Doxorubicin as “Standard of Care” for treatment

Treatment with Doxorubicin clearly diminished tumor growth in both 4T1 breast cancer models and can therefore serve as a potent positive control here (Fig. 2).

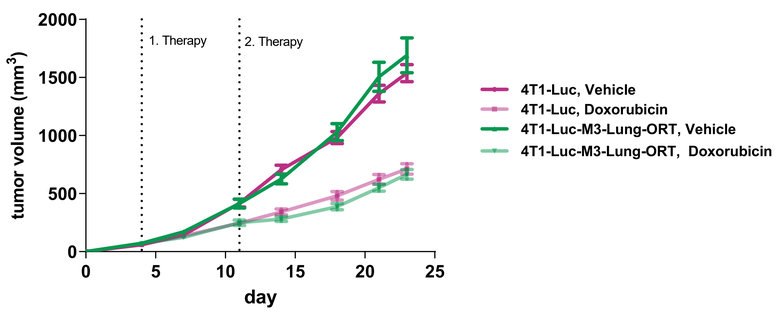


Fig. 2: In vivo growth characteristics of two different 4T1-Luc cell lines in mice treated with Doxorubicin or vehicle control

Metastasis capabilities of the new 4T1 Metastasis model

The new 4T1-luc-M3-lung-ORT metastasizing cells were implanted ortotopically in parallel to the parental 4T1-luc cell line and we analysed various organs ex vivo for luciferase activity. The luciferase acticity is the read out for metastasis here. In several organs a much higher activity was obtainable in the metastasis cell line, 4T1-luc-M3-lung-ORT, compared to the parental cell line (Figs. 3 and 4). Only in liver tissue no metastases could be detected in both models.

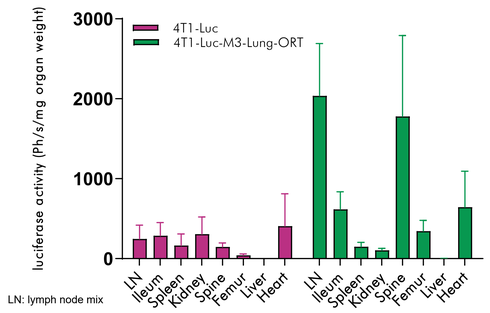


Fig.3: Ex vivo analysis of luciferase activities of various organs of mice implanted orthotopically with 4T1 cells, mean values +/- SEM

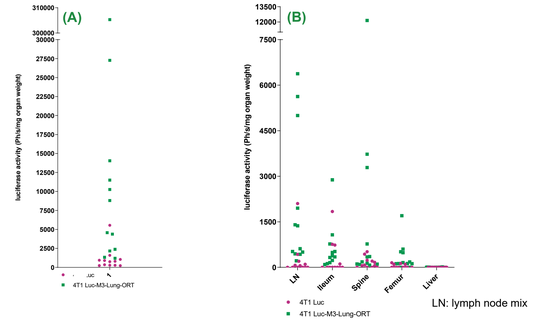
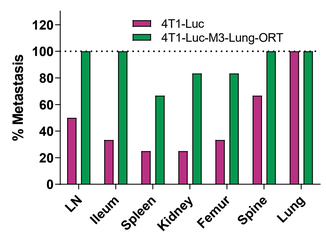


Fig.4: Ex vivo analysis of luciferase activities of various organs of mice implanted orthotopically with 4T1 cells, single values, (A) in lung, (B) in other organs

Whereas in the parental model only in lung tissue metastasis in all implanted mice (12/12) was detectable, the metastasis rate in the new model, generated by “subpopulationing”, bounced up to 4 organs with measurable metastasis in 100 % of the mice, and in general to a much higher degree of metastasis in most other organs (Fig. 5).



LN: lymph node mix

Fig. 5: Metastasis rates in different organs of mice implanted with 4T1 cells

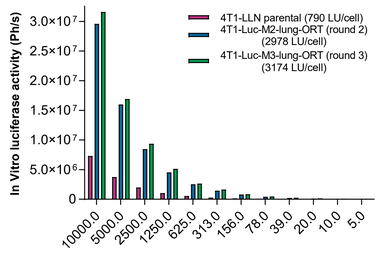


Fig. 6: In vitro luciferase activities of different 4T1 cells

Analysis of “In vitro luciferase activity” revealed an increase of activity in the metastasis cell lines (round 2 and 3) compared to the parental cell line (Fig. 6). Thus, these cell lines allow a much higher sensitivity when detecting metastases.

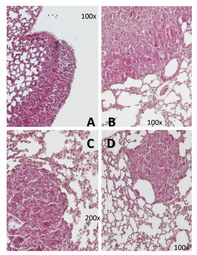


Fig. 7: HE-stained sections of FFPE samples of lungs containing metastases derived from 4T1-Luc (A, B), or 4T1-Luc-M3-Lung-ORT (C, D) tumors.

No obvious differences in appea-rence, size or number of metas-tases have been observed com-paring both models (Fig. 7).

Outlook

With this new 4T1-M-ortho cell line we have available a metastazing breast cancer cell line that shows reliable metastasis observed in several organs of the mouse when implanted orthotopically. This model is suitable for testing potential anti-metastasizing compounds aginst breast cancer. In addition, since this model is established in immunocompetent mice, and is therefore also perfectly suited for approaches involving the immune system of breast cancer patients.

Acknowledgement

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