Abstract #B164

A platform of metastatic bioluminescent PDX: Focus on HBCx14-Luc1 metastatic bioluminescent breast tumorgraft model for preclinical studies

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INTRODUCTION AND RATIONALE

- The ability of tumor cells to spread and form metastases is one of the key parameters used to classify tumor aggressiveness. Metastasis detection at diagnosis or during follow-up post-surgery is associated with poor prognosis. The paucity of pertinent models to investigate the biology of metastasis renders problematic the set-up of anti-metastasis therapies. We took advantage of our human patient-derived xenograft (PDX) collection to develop models suitable to assess anti-metastatic therapy.
- In order to identify metastatic PDXs, ex vivo studies with 5-Aminolevulinic acid (5-Ala) were performed. 5-Ala is a porphyrin precursor that is preferentially metabolized into porphyrin by tumor cells (1), The use of this technique allowed the observation that metastases developed in a variety of PDX models from different organ of origin (breast, liver, prostate, pancreas and skin) (Figure 1 and Table 1).
- For all models that showed a metastatic phenotype, SCID mice proved more permissive to metastasis development than nude mice. Therefore, as PDX growth on nude mice minimizes the risk of contaminating human tumors with murine lymphoma, we decided to keep PDX passages on nude mice and graft tumors on SCID only to perform metastasis induction experiments.
- To enable evaluation of metastasis response to treatment, we labeled tumors with lentivirus-mediated luciferase insertion, allowing longitudinal follow-up of metastasis formation by non-invasive imaging. A review of our metastatic bioluminescent PDX panel is described here with a particular focus on the heterogeneity of the metastatic spread in different PDXs, and on the impact of primary tumor resection in a well-characterized bioluminescent triple-negative breast cancer model, HBCx-14-Luc1.

RESULTS

- At present, 5 PDXs shown to be metastatic by *ex vivo* 5-Ala assay have been transduced to express luciferase. and analyzed as listed in Table 2. For all models, metastasis detection assay has been performed on mice grafted with bioluminescent tumor specimens, and not by cell injection, in order to be sure that metastasis development is the result of tumor cell migration from the primary tumor. All PDXs developed bioluminescent metastasis with model-dependent delay of occurrence. By *in vivo* imaging, we could only detect lung metastasis. By contrast, *ex vivo* bioluminescence assay showed that, in addition to lungs, several other organs were also colonized by the tumor in a model-dependent way. Particularly, a Her2-positive breast PDX model, HBCx-5-Luc2, induced bioluminescent metastases at pancreas, brain, spleen, liver, lung and bones (Figure 2), whereas metastatic spread in triple-negative breast cancer model HBCx-12B seem to be rather specific at brain and liver.
- We decided to evaluate the impact of primary tumor surgical removal on metastasis formation. To this aim, we chose the triple-negative breast cancer model HBCx-14-Luc1, one of our bioluminescent models best characterized in terms of profile of metastasis occurrence and stabilization of bioluminescence intensity in tumor cells. To investigate the influence of primary tumor resection, we monitored both lung metastasis frequency and delay of occurrence in resected and unresected mice. As shown in Table 3, no difference was observed between resected (7/8 positive at D88) and non-resected mice (11/13 at D93) when looking at metastasis positive mice at end point. However, while the first day of metastasis occurrence was similar between non-resected (D48) and resected mice (D53), the number of metastasis positive mice at this early time-point was higher for the non-resected (7/8) compared to resected mice (3/18). The graphs in Figure 3 show the impact of primary tumor resection on tumor growth and metastasis development.

CONCLUSIONS AND PERSPECTIVES

- Bioluminescent metastatic PDX models derived from tumors of different histotype were successfully established by growing luciferase-engineered PDXs subcutaneously into appropriate immunodeficient mouse strains. Additional PDX models have been transduced and are currently being screened *in vivo* and *ex-vivo* in order to get model-specific patterns of metastatic spreading.
- So far, only lung metastasis could be detected in vivo. This is probably due to the weakness of the signal from metastasis. As metastasis development is a long term process, the most important limitation to the analysis of metastasis occurrence is the rapidity of primary tumor growth, which frequently reaches the volume indicated for ethical sacrifice in few weeks.
- The observation that primary tumor resection delays but does not prevent metastasis formation allows to keep mice under observation for a longer time. This approach will put the assay closer to the clinical situation, and will hopefully allow pushing metastasis growth to a size that can be detected in vivo, thus rendering possible live observation in various organs of metastasis response to anti-metastatic agents.
- These models will provide a useful tool to explore the biology of metastasis and evaluate anti-metastatic therapies in a selection of organs. Our goal is to extend as much as possible the number of bioluminescent metastatic PDX models available for research, in order to reproduce the heterogeneity of metastatic tumor behavior that is observed in patients.

REFERENCES: (1) Murayama Y, et al. Precise detection of lymph node metastases in mouse rectal cancer by using 5-aminolevulinic acid. Int J Cancer. 2009 Nov 15;125(10):2256-63.

MATERIALS AND METHODS

- All *in vivo* studies were conducted in conformity with National Veterinarian Regulations as defined by the French Ministry.
- PDX collection screening for identification of metastatic models was performed by *ex vivo* fluorescence. For imaging, protoporphyrin IX precursor 5-Ala (250 mg/kg) was injected intravenously, and 4h later tumors and organs (lungs, kidneys, spleen and brain) were harvested and checked for accumulated protoporphyrin IX fluorescence. Positive regions underwent histological analysis to confirm the presence of metastasis.
- Tumor cells from freshly excised PDXs were dissociated using GentleMACS™Dissociator (Miltenyi Biotec) and enzymatic cocktail (Collagenase, Hyaluronidase and DNase) (Sigma). To remove dead cells and debris, cells were washed three time, then counted and cell viability was assessed with trypan blue (Invitrogen).
- Cells were transduced with lentiviral vector particles containing firefly luciferase gene (Vectalys). One million transduced cells were injected subcutaneously into immunocompromised nude (Athymic Nude-*Foxn1^{nu}*) or SCID (severe combined immunodefeciency : CB17/IcrHsd-Prkdcscid) mice. Tumor growth and metastasis formation were monitored throughout passages by whole body visualization and *ex vivo* organ imaging at the end point using the Xenogen IVIS® Lumina. Before *in vivo* imaging, mice were injected with Luciferin solution at 150 mg/kg.
- To study the influence of the primary tumor on metastatic spread and to prolong the follow-up for metastasis detection primary tumor resection was performed when tumor size reached 500 mm^3.
- *In vivo* bioluminescence was measured every two weeks, over 8 to 20 weeks, to assess metastasis appearance and progression. The presence of metastases was confirmed by histological analysis.
- *Ex vivo* bioluminescence of tumor and organs was checked using a 150 µg/ml luciferin bath.

FIGURES AND TABLES

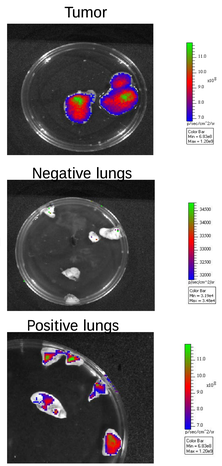

Figure 1
Metastasis detection visualised by fluorescence induced by 5-Ala metabolization

Table 1
List of metastatic PDXs identified by 5-Ala assay.

Tissue of origin	5-Ala-positive PDXs
Breast (n=14)	HBCx-5 HBCx-6 HBCx-7 HBCx-9 HBCx-12B HBCx-14 HBCx-19 HBCx-23 HBCx-28 HBCx-30 HBCx-33 HBCx-34 HBCx-39 T330
Liver (n=2)	HB-234-PAL HB-244-MAN-RED-239
Lung (n=3)	IC14LC18 ML1LC2-MAR IC15LC18-JAM
Pancreas (n=1)	PANx-005
Prostate (n=2)	HID28 PAC120
Skin (n=3)	MELx-006 MELx-008 MELx-009

Table 2
List of bioluminescent PDXs analyzed for metastatic spreading

PDX Model	Primary tumor resection	N of mice	Luciferase-positive metastasis at endpoint							
			mean latence (days)	In vivo	ex-vivo					
				Lung	Lung	Liver	Spleen	Pancreas	Brain	Bones
HBCx-12B-Luc3	No	10	50+/-12	10/10	4/4	1/4	0/4	0/4	2/4	0/4
HBCx-5-Luc2	No	17	83+/-40	16/17	17/17	6/12	1/13	8/13	11/13	10/13
HBCx-14-Luc1	Yes	16	75+/-8	8/16	10/16	NA	NA	NA	NA	NA
PAC120-Luc	No	6	65	1/5	4/5	NA	NA	NA	NA	NA
PANx-005-Luc1	No	11	42	6/11	11/11	0/11	1/11	0/11	0/11	1/11

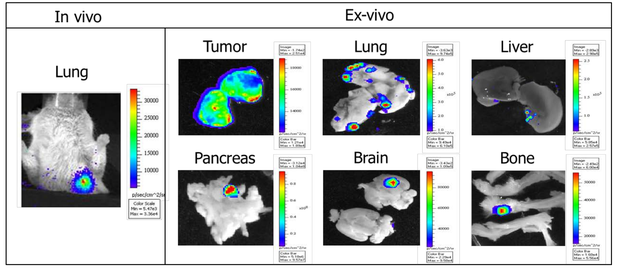

Figure 2
Imaging of HBCx-5-Luc2 metastases detected in vivo and ex-vivo

Table 3
Comparison of metastatic occurrence delay and frequency in HBCx-14-Luc1 mice with unresected or day 40-resected primary tumors

PDX Model	N of mice	unresected resected at day 40	First metastasis observed (days)	N of mice with lung metastasis at 1 week after first apparison	mean latence (days)	Luciferase-positive lung metastasis at endpoint	
						In vivo	ex-vivo
HBCx-14-Luc1	26	8 18	48 53	7/8 3/18	49 +/-2 66 +/-15	7/8 11/13	7/8 11/13

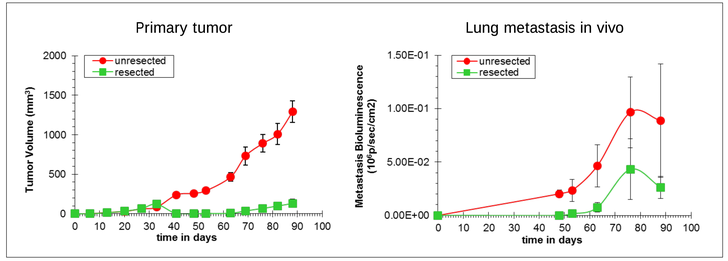

Figure 3
Graphical representation of tumor and metastasis growth in mice with resected and unresected primary tumors