AACR-NCI-EORTC 2015

Cell-autonomous activation of the Interferon/STAT1 pathway in response to genotoxic treatment

Poster #A103
Booth #506

Julie Gaston[1,2] , Laura Cheradame[1,2], Marie-Emmanuelle Legrier[3], Olivier Déas[1], Marie-France Poupon[2], Jean-Gabriel Judde[2], Vincent Goffin[1] and Stefano Cairo[2]

[1]Inserm 1151/Institute Necker Enfants Malades (INEM), Paris , France, [2]Xentech, Evry, France, [3]Institut Curie, Paris, France

Abstract

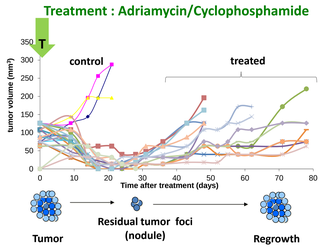
Identification of the mechanisms of tumor chemoresistance remains an unmet need in oncology. Among the reasons for this challenging issue is the lack of reliable preclinical models that represent inter and intra-patient tumor heterogeneity observed in the clinic. Patient-derived xenografts (PDXs) consist in grafting human cancer samples into immunocompromised mice directly after patient surgery. Since each PDX faithfully recapitulates patient's tumor genetics, these preclinical models well represent the intrinsic heterogeneity of cancer. We took advantage of the large collection of breast cancer PDXs held at XenTech to address the mechanisms of tumor response to treatment vs tumor recurrence. Upon receiving chemotherapy, some breast cancer PDXs (the 'responders') undergo tumor shrinkage, whereas others continue to grow (the 'non-responders'). Comparative transcriptomic profiling of laser-microdissected cancer cells showed that the reduction of tumor volume in responders was closely associated to the over-expression of genes related to the interferon (IFN)/signal transducer and activator of transcription 1 (STAT1) pathway. Using mouse versus human cytokine arrays we observed that activation of this pathway was associated with the secretion of ligands of human origin (cancer) rather than with ligands secreted from the nude mice residual immune system. Next, we screened a large number of cell lines to identify in vitro cell models able to mimic cell-autonomous induction of the IFN/STAT1 signature after genotoxic treatment. Both an immortalized cell line and a primary culture dissociated from a responder PDX were shown to activate the IFN/STAT1 pathway and to express the cognate gene signature after treatment with mafosfamide (the active metabolite of cyclophosphamide used in the clinic). Expression analysis (qPCR) confirmed induction of IFN type I in cells in vitro. Conditioned medium collected from mafosfamide-treated cancer cells was able to activate luciferase reporter genes harboring ISRE (interferon stimulated response elements) and GAS (gamma interferon activated sequence) response elements, meaning that active ligands of the IFN/STAT1 pathway were secreted. Accordingly, STAT1 gene silencing (siRNA) resulted in markedly attenuated gene signature expression after mafosfamide treatment. The addition of conditioned medium significantly reduced mafosfamide-induced cancer cell death suggesting that the over-expression of the IFN/STAT1 pathway may ultimately have protective effect on cancer cell viability. In conclusion, this study supports that cell-autonomous activation of the IFN/STAT1 pathway is a surrogate biomarker of initial tumor shrinkage in response to genotoxics, and suggests that it may play a role in tumor resistance to treatment.

Introduction

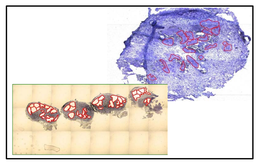
Triple negative breast cancer (TNBC) is a very aggressive subtype of breast cancer. It lacks expression of estrogen (ER) or progesterone (PR) receptors and over-expression of HER-2, hence it cannot benefit from current targeted therapies. Its metastatic propensity is higher than in other types of cancer. After chemotherapy, **recurrence** is frequently observed and is most often lethal for the patient. Xentech has developed a panel of Patients Derived Xenograft models (**PDXs**) from TNBC in order to study and better understand the mechanisms of response to treatment and recurrence.

PDXs analysis

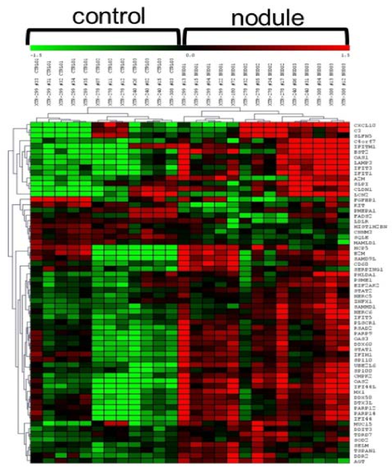
Treatment : Adriamycin/Cyclophosphamide



Laser microdissection (LMD)

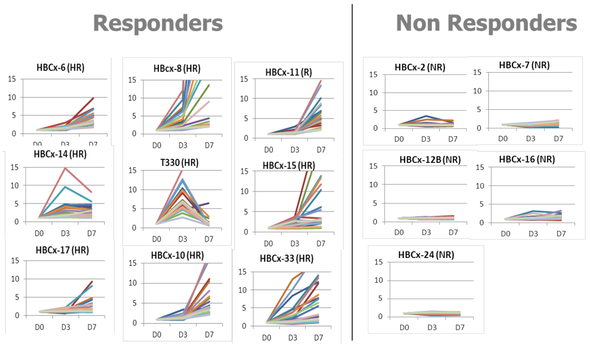


Transcriptome analysis: nodule vs control



Identification of a 21 gene SIGNATURE

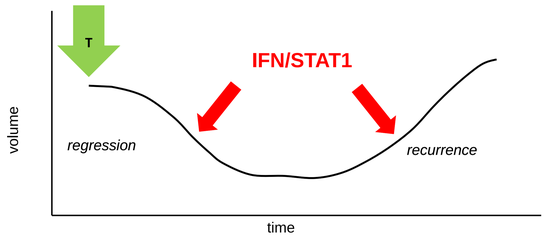
- **IFN/STAT1 pathway**: STAT1, STAT2, IFIT1, IFITM1, IFI44, OAS1…
- **Other pathways**: LAMP3 (autophagy) / PARP12, PARP9, PARP14 (DNA repair)



- **Signature observed ONLY in tumors responding to treatment**
- **Detectable 3 to 7 days after A/C, i.e. BEFORE the nodule phase**

Working Hypotheses

Is the IFN/STAT1 pathway involved in tumor regression, tumor recurrence, or both?

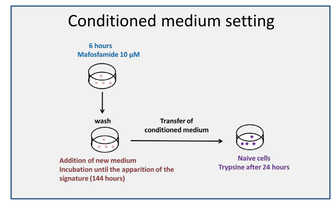


Objectives and Experimental Strategy

Identify *in vitro* models that mimic the *in vivo* observations (PDX)
- Cell line screening

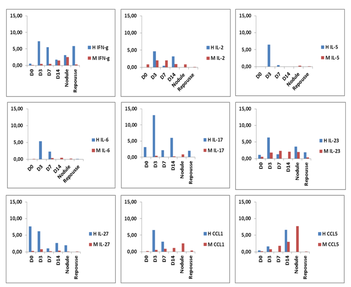
Determine the mechanism of induction of the IFN/STAT1 signature
- Identify upstream triggers (expression profile, conditioned media, reporter genes)
- Decipher intracellular pathways

Determine the role of the signature in tumor response to treatment vs recurrence
- Target gene silencing (siRNA)
- Readout : viability assay (10 – 50 days post-treatment)



Results

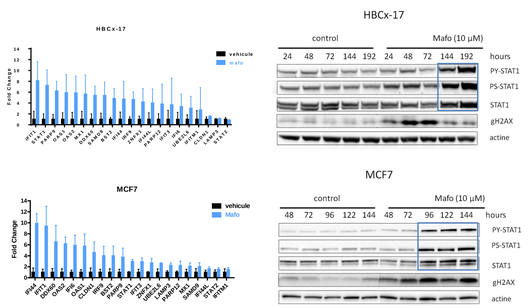
Cytokine array (PDX)



- Cytokine candidates upstream of the IFN/STAT1 pathway are mostly **expressed by human (cancer)** rather than mouse (stroma) cells.
- Most cytokines are induced **shortly (few days) after treatment** (D3-D7)

Development of *in vitro* models

To confirm the cancer cell-autonomous induction of the IFN/STAT1 pathway after chemotherapy, a screening of *in vitro* cell lines was performed.



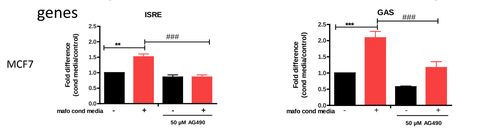
HBCx-17, a primary culture established from a TNBC PDX, and **MCF7**, the most widely used ER-positive breast cancer cell line, **induce the IFN/STAT1 pathway after genotoxic treatment *in vitro*.**

Paracrine IFN/STAT1 pathway induction

Stimulation of naïve MCF-7 cells by conditioned media induces:

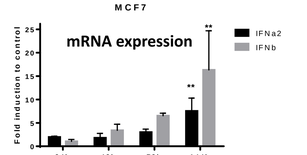
1. STAT1 tyrosine phosphorylation

2. Transcriptional activation of ISRE and GAS luciferase reporter genes

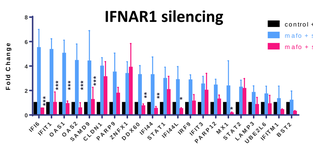


ISRE and GAS are Stat1/2 response elements found in the promoters of IFN-stimulated genes

Type I IFNs as candidates

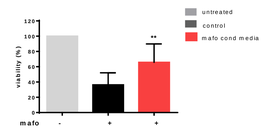


Cell-autonomous induction of type I IFN expression after chemotherapy

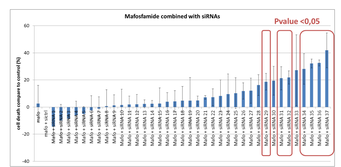


Type I IFN receptor (IFNAR1) plays a central role in the induction of the IFN/STAT1 signature

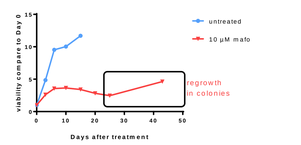
Role of the IFN/STAT1 pathway in cancer recurrence



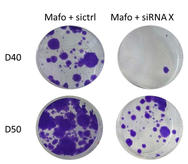
Conditioned media protect cells against genotoxic stress



siRNA screening identifies **individual IFN/STAT1-regulated genes** whose inhibition potentiates the effect of genotoxics



Colony growth assay after 20 days of treatment with mafosfamide.



Colony assays: **target silencing delays recurrence**

Conclusions

- **In response to chemotherapy:**
  - **Breast cancer cells produce several "immune cytokines" in a cell- autonomous manner.**
  - **Among these, type I IFNs are likely major triggers of the IFN/STAT1 pathway in cancer cells**
- **IFN/STAT1 pathway activation induced by genotoxics may be both**
  - **a predictive marker of response to treatment (at the signature level)**
  - **Involved in the persistence of residual cells facilitating cancer recurrence (at the individual gene level)**

Acknowledgements

- The authors gratefully acknowledge the scientific and technical staff from XenTech and INEM U1151, in particular: Sophie Banis, Vanessa Yvonnet, Myriam Lassalle, Enora Le Ven, and Lucila Sackmann Sala.