The role of Toll Like Receptor 9 (TLR9) in breast cancer

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**TLR9** dsDNA

**FACTS on TLR9**

Highly expressed on immune cells (pDC and B cells human)

Weakly expressed on epithelial cells (skin and cervix human)

Activated by dsDNA

Expressed in the ER - shifts to endosome

Breast cancer ¹

Immune system

Cellular transformation
TLR9 pivotal in immune responses and cell cycle control

**Immune response** 1, 2, 3, 4
Type I IFN by pDC

**Oncogenic stress** 5, 6, 7, 8

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5. **Proliferation**, Colorectal cancer, Si Ming Man *et al.*, Cell 2015
Question

Role of TLR9 in Breast Cancer?

Methods

5 WPs

Objective 1. TLR9 expression
Cohort (Kings College London)

Objective 2. Mechanism
BC human *in vitro and ex vivo* models

Objective 3. Mechanism
BC murine *in vivo* models
TLR9 in BC tumour cells

[Image of histological section with labels: Tu (tumor), TME (tumor microenvironment).]

TLR9 tumor
Team 1 UH
KCL

TLR9 pDC
TEAM 2 NVB
TEAM 3 OT
Objective 1. TLR9 expression
Cohort (Kings College London)

30 sections

Normal tissue score =1
DCIS /Invasive tissue =0

Levels of TLR9 expression

NORMAL vs. DCIS_INVASIVE logistic regression model
odds ratio = 0.16 (normal is the reference)
TLR9 expression is blocked in several virus-induced cancers

**Ex vivo**

NORMAL vs. DCIS_INVASIVE: logistic regression model
odds ratio = 0.16 (normal is the reference)

**In vitro**

RESULTS

http://www.broadinstitute.org/ccle/home
http://brainarray.mbnl.med.umich.edu/Brainarray/Database/
Objective 2.
Mechanism

BC human in vitro models

• Proliferation: colony assay

• SASP (Senescence-Associated Secretory Phenotype)

• Tumour suppressor
RESULTS: Proliferation is blocked by TLR9 in breast cancer cells

TLR9

M361TLR7

10
100
1000

Dox

TLR9

Dox

TLR7

% of S-G2 phase

-DOX

+DOX

0
10
100
1000

Time (d)

Cumulative cell number

-TLR

+TLR

-TLR7

+TLR7

Dox

Dox

Dox

Dox
RESULTS: TLR9 induces tumour suppressors and SASP

DNA damage, hypoxia, cellular stress, oncogene, radiation, other factors ...

- +. DOX

p53
p16
actin

p16INK4a
p21cip

CDK dependent

Stop cell cycle

Cell senescence

β-gal

TLR9 +

IL-6 IL-8

CXCL-1

No dox
Dox

5% CO2 à 37°C
3 jours

Cytokines

Expression of tumour suppressors
DNA damage, hypoxia, cellular stress, oncogene, radiation, other factors ...

p53
p16
actin

p16INK4a
p21cip

CDK dependent

Stop cell cycle

Cell senescence

β-gal

TLR9 +

IL-6 IL-8

CXCL-1

No dox
Dox

5% CO2 à 37°C
3 jours

Cytokines

Expression of tumour suppressors
CONCLUSIONS and next steps

- **Senescence**
- **Slow down in cell cycle**
TLR9 immunity in BC
pDC are specialized in antiviral responses via type I IFN production.
pDC at the center of an immunosuppressive microenvironment in breast and ovarian cancers

Premalignant lesion → Advanced oncogenesis → Tumor growth

**Elimination** → Immuno-surveillance

**Equilibrium** → Immuno-selection

**Escape** → Immuno-subversion

Inhibitory and non-IFN-I inducing endogenous activating stimuli

IFN-α

Treg expansion

Th2 differentiation

Labidi-Galy et al, Can Res 2011
Sisirak et al, Can Res 2012
Faget, Can Res 2012
Sisirak et al, Int J Cancer 2013
Ghirelli et al, Can Res 2015
Do pDC play a dual role in breast cancer immunity?

Any role for pDC and type I IFNs?

IFN-α producing TApDC

Inhibitory and non-IFN-I inducing endogenous activating stimuli

Elimination

Premalignant lesion

Advanced oncogenesis

Tumor growth

Equilibrium

Immuno-surveillance

Immuno-selection

Immuno-subversion

Tumor cells

pDC

Altered TApDC

Labidi-Galy et al, Can Res 2011
Sisirak et al, Can Res 2012
Faget, Can Res 2012
Sisirak et al, Int J Cancer 2013
Ghirelli et al, Can Res 2015
Do pDC play a dual role in breast cancer immunity? What is the role for TLR9?

- Premalignant lesion
- Advanced oncogenesis
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Elimination → Immuno-surveillance → IFN-α → IFN-α producing TApDC

Equilibrium → Immuno-selection

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TLR9?

Any role for pDC and type I IFNs?

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Faget, Can Res 2012
Sisirak et al, Int J Cancer 2013
Ghirelli et al, Can Res 2015
Objective 1. TLR9 expression

Cohort (Kings College London and CLB, Lyon)

TLR9 is strongly expressed in the microenvironment of human breast tumors

Vey, Mussard et al, in preparation
Do pDC play a dual role in breast cancer immunity? What is the role for TLR9?

**Diagram:**
- Premalignant lesion → Advanced oncogenesis → Tumor growth
- **Elimination** → **Equilibrium** → **Escape**
- **Immuno-surveillance** → **Immuno-selection** → **Immuno-subversion**
- TLR9?

- TLR9 inducing endogenous activating stimuli
- Inhibitory and non-IFN-I inducing endogenous activating stimuli
- IFN-α, Treg expansion, Th2 differentiation
- Labidi-Galy et al, Can Res 2011
- Sisirak et al, Can Res 2012
- Faget, Can Res 2012
- Sisirak et al, Int J Cancer 2013
- Ghirelli et al, Can Res 2015
BAD-LAMP controls TLR9 trafficking and signaling in human pDC

Objective 2. Mechanism
BC human *in vitro* and *ex vivo* models

BAD-LAMP & TLR9

**NS** 1h CpGA 24h CpGA

BAD-LAMP downmodulation

BAD-LAMP overexpression

Combes et al, Nature Communications 2017
BAD-LAMP expression is enhanced in breast tumor pDCs and its downregulation is prevented by inhibitory tumor supernatants.

Objective 2.
Mechanism

BC human *in vitro* and *ex vivo* models

A  Blood patients’ pDC

B  Breast Tumor pDC

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Combes et al, Nature Communications 2017
Do pDC play a dual role in breast cancer immunity? What is the role for TLR9?

Any role for pDC and type I IFNs?

IFN-α producing TApDC

[pDC] Tumor cells

neutrophil

TLR9?
Objective 2.
Mechanism

**BC human in vitro and ex vivo models**

Vey, Mussard et al, in preparation
Evidence for the activation of type I IFN pathway in human breast tumors

Vey, Mussard et al, in preparation
Endogenous TLR agonists are present in breast tumors and are able to activate or potentiate pDC activation in vitro.

Objective 2. Mechanism

BC human in vitro and ex vivo models
Conclusion and next steps

• **Further characterize the intratumor endogenous TLR ligands**
  - Mitochondrial DNA vs Genomic DNA
  - Demonstrate the role of TLR

• **Further characterize immune infiltrate and immune pathways (TLR9, IFN) dominating in early breast cancers versus invasive breast cancers in patients**

• **Demonstrate the role of pDC and neutrophils in breast tumor immunosurveillance in vivo by depleting experiments**

Joint conclusion

**TLR9 tumor**
Team 1 UH KCL
- Expression is lost tumour cells
- Slows down proliferation
- Induces senescence

**TLR9 in BC**

**TLR9 TME**
TEAM 2 NVB TEAM 3 OT
- Strong expression in tumor pDC
- Activation blocked by BAD-LAMP
- Endogenous ligands are present in BC

Figure 3 - uploaded
Ahmad
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Mala Maini, UK
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