Respuesta inmune y expresión de RNA en los pacientes operados de TB

Dr. Cris Vilaplana
cvilaplana@gmail.com, cvilaplana@igtp.cat
https://unitatdetuberculosiexperimental.wordpress.com/
We do not know what happens inside

No correlators of clearance/ sterilization)

The **SH-TBL PROJECT**: We study TB lesions from surgery patients
Hypothesis

1. on the role of the host in the mechanisms associated to the generation and evolution of active TB and
2. about future diagnostic and/or prognostic biomarkers of TB disease.

ClinicalTrials.gov NCT02715271
Prospective substudy

Candidate to receive therapeutical surgery → Pre-Surgery → Surgery → Post-Surgery (Discharge)

Peripheral Nodulus (N)
Cavity Center (C)
Cavity Internal Wall (I)
Cavity External Wall (E)
Healthy nearby tissue (H)

For each lesion’s zone, a piece of approx. 0.5x0.5x0.5 cm for pathology
Host:

- Pathology: UTE + Tanvier Omar, SA
- Clinical, radiological and epidemiological data: UTE
- Health Quality of Life: UTE
- Biomarkers: UTE (in blood, urine and host transcriptomics- this + Imperial College London)

Pathogen:

strain sequencing (Iñaki Comas, Valencia)
Microbe expression (Simon Waddell, UK)

Data integration: Alex Rosenthal (NIAID/NIH)
Assessment of immune markers in blood and urine

Methods

36 microbiologically cured, Georgian patients after completion of ATT
Analysis of immune markers with LUMINEX and ELISA
Surgery – lesion resection
• Culture
• Macroscopic characterization

Microbiology of lesion

<table>
<thead>
<tr>
<th>Culture</th>
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<td>+</td>
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<table>
<thead>
<tr>
<th>Response</th>
<th>Culture</th>
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<tbody>
<tr>
<td>Fast</td>
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<tr>
<td>Slow</td>
<td>1</td>
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Drug resistance

<table>
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<tr>
<th>Drug resistance</th>
<th>Culture</th>
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<tr>
<td>Sensitive</td>
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</tr>
<tr>
<td>Resistant</td>
<td>0</td>
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Lesion phenotype & immune markers

Not fresh necrosis
Fresh necrosis

Gender
Alcoholism
Smoking habits
BMI
Tuberculosis symptoms
Relapse patients

...
We find differences depending on gender, size of lesion, type of necrosis, alcohol consumption.

First footprints towards a PROGNOSIS immune marker panel
Human tuberculosis lung granuloma zones and blood collection by timepoints

TISSUE SAMPLES
- Peripheral Nodulus (N)
- Cavity Center (C)
- Cavity Internal Wall (I)
- Cavity External Wall (E)
- Healthy nearby tissue (H)

BLOOD SAMPLES
- Pre surgery blood
- Post surgery/discharge moment blood

TOTAL-RNA RNA-seq
- Illumina
- Read: paired-end
- Read length: 2x100bp
- Platform: Illumina HiSeq4000

Imperial College London
Healthy lung tissue as a control: A proof of concept

Healthy lung parenchyma have a different RNA expression pattern from granuloma’s signature

Granuloma vs Healthy tissue heatmap
Top 50 padj <0.05 values
Tuberculosis granuloma expression pattern from healthy lung tissue

Granuloma zones (individually expressed) vs Healthy lung parenchyma
Venn diagram (padj <0.05)

Human tuberculosis granuloma is spatial-transcriptomically differentiated. The closer to healthy tissue, less unique genes encountered.
Gender differences in granuloma expression pattern

Gender seems to play an important role in granuloma’s expression

Granuloma zones and healthy parenchyma by gender
differential expression
Top 50 padj < 0.05 values
And much more variables are still to be assessed...

1. Tissues
2. Granuloma spatial differential expression
3. Gender
4. Age
5. Body Mass Index
6. Alcoholism
7. Smoking habits
8. Tuberculosis symptoms
9. Relapse patients
10. Drug sensitivity
11. Fresh necrosis
12. Culture conversion
13. Cavitation
14. AFB granuloma samples
15. AFB by patients
16. Lesion localization
17. Lesion size

The genetical expression pattern of all these variables may be linked to a worse patient’s prognosis...

Potential PROGNOSIS SIGNATURE
It is necessary to check, compare and validate these results with genetic profiles found in TB lesions!
Human tuberculosis granuloma lesion as a source of blood signatures validation

Discordance and Concordance of Transcriptomic Response (DISCO)
(Domaszewska, T, 2017)

disco.score := \left| \log FC_1 \right| \cdot \left| \log FC_2 \right| \cdot \left( \left| \log_{10}(p_1) \right| + \left| \log_{10}(p_2) \right| \right) \cdot \text{sign}(\log FC_1) \cdot \text{sign}(\log FC_2)

Calculates the concordance/discordance score between two heterologous data sets (different tissue sources, technical platforms and/or organisms)

Granuloma transcriptomical signature

Patient is cured after treatment (WHO), but still needs surgery!

Prognosis related transcriptomical signatures obtained from peripheral blood (GEO public datasets)

Ottenhoff 2012
Bloom 2012
Bloom 2012
Thompson 2017*
Ottenhoff 2012
Bloom 2012

Baseline (no treatment)
Active TB patients

Week 24 (treatment done)
*(Cured vs No Cured)
Human tuberculosis granuloma lesion as a source of blood signatures validation: Biomarkers

Concordance/Discordance plots (disco.score by DISCO package)

Baseline vs week 24

Baseline vs week 24

Baseline vs week 28

Baseline vs month 12

**Concordance** = similarity between granuloma genes at baseline (no treatment started)

**Discordance** = similarity between granuloma genes at treatment finished timepoint
Human tuberculosis granuloma lesion as a source of blood signatures validation: Biomarkers

Concordance/Discordance plots (disco.score by DISCO package)

Baseline vs week 24

Baseline vs week 24

Baseline vs week 28

Baseline vs month 12

Granuloma logFC cutoff= +1.5

DISEASE related genes

PROGNOSIS related genes
We have done a pathology classification consensed by Pr. Omar (Witts Univ, Johannesburg) and validated for the first 10 patients.

We are now recording all the variables for all the patients.

We have analyzed all the data.
Now we are doing the graphs to depict all the variables.
WE ARE ALWAYS HAPPY TO COLLABORATE!
PLEASE SEND ME AN EMAIL AT
cvilaplana@gmail.com
THANKS!!!