BUSTA N. 1

DOMANDA N. 1 Back up e protezione dei dati: rilevanza nelle attività di ricerca (max 20-25 righe).

DOMANDA N. 2 Citofluorimetria multiparametrica: descrizione di una tecnica (max 20-25 righe).

Firma della commissione

BUSTA N. 2

DOMANDA N. 1 Strumentazione di laboratorio: calibrazione e controlli di qualità nei laboratori di ricerca (max 20-25 righe).

DOMANDA N. 2 Tecniche di immunofluorescenza e immunoistochimica di cellule e tessuti (max 20-25 righe).

Firma della commissione

BUSTA N. 3

PROVA ESTRATTA

DOMANDA N. 1 Colture cellulari: prevenzione delle contaminazioni (max 20-25 righe).

DOMANDA N. 2 Tecniche “Omiche” nella ricerca: descrizione di una tecnica (max 20-25 righe).

Firma della commissione

DOMANDE

1. Quali sono le classi di sicurezza di cappe biologiche
2. Descrivi la metodica di ELISA
3. Descrivi la metodica di Immunofluorescenza su cellule e tessuti
4. Descrivi metodiche di immunoistochimica
5. Descrivi i metodi di estrazione del DNA
6. Descrivi i metodi di estrazione dell'RNA
7. I terreni più utilizzati nelle colture cellulari di cellule tumorali
8. Descrivi la metodica della Polymerase Chain Reaction (PCR)
9. Descrivi la metodica del Western Blotting (WB)
10. Descrivi la metodologia della citofluorimetria a flusso
11. Descrivi la separazione di cellule del sistema immunitario dal sangue periferico
12. Descrivi la tecnica di ibridazione in situ (FISH)

13. L'elettroforesi delle proteine

CANCER RESEARCH
2022 Sep 16;82(18):3291-3306. doi: 10.1158/0008-5472.CAN-22-1427.

Lipid-Associated Macrophages Are Induced by Cancer-Associated Fibroblasts and Mediate Immune Suppression in Breast Cancer

Eleonora Timperi[1], Paul Gueguen[1], Martina Molgora[2], Ilaria Magagna[3], Yann Kieffer[3], Silvia Lopez-Lastra[1], Philemon Sirven[1], Laura G. Baudrin[4], Sylvain Baulande[4], Andr_e Nicolas[5,6], Gabriel Champenois[5,6], Didier Meseure[5,6], Anne Vincent-Salomon[5,6], Anne Tardivon[7], Enora Laas[8], Vassili Soumelis[9,10], Marco Colonna[2], Fatima Mechta-Grigoriou[3], Sebastian Amigorena[1], and Emanuela Romano[1,11]

1

Breast cancer patient cohorts and tissue specimens

Human sample collection and use for the studies were approved by Institutional Review Board and Ethics committee of the Institut Curie (Paris, France; Feb 12, 2014) and Commission Nationale de l'informatique et des Libert_es (CNIL; N. approval: 1674356; SCANDARE, NCT03017573), and the national ethics committee. The studies were conducted in accordance with the recognized ethical guidelines of Helsinki Declaration. Tumor (TUM) and adjacent juxta-tumor (JT) tissue samples were from early-stage,

2

treatment-na€_ve TNBC (N ¼ 32), and treatment-na€_ve luminal breast cancer (LBC; N ¼ 21) patients. Normal mammary tissues (N ¼ 2) from prophylactic mastectomies were included in the study. For all the patients included in the study we obtained written informed consent. Invasive ductal carcinoma of the TNBC histology was defined by estrogen receptor (ER), progesterone receptor (PR), and, ERBB2 (HER2/Neu) negativity; LBC was defined by positive immunostaining for ER and/or PR. The cut-off used to define hormone receptor positivity was 10% of stained cells according to Cardoso and colleagues (21).

3

For scRNA-seq studies, fresh TUM specimens from treatment-na€_ve TNBC (N ¼ 4) patients were obtained, of which, 3 were paired with JT samples. Normal mammary gland, TUM, and JT specimens both from patients with treatment-na€_ve TNBC (N ¼ 29) and LBC (N ¼ 21), were included in the flow cytometry (FC) analysis. Buffy coats from healthy donors (HD) were obtained from Etablissement Fran¸cais du Sang (Paris, France). Blood from patients with treatment-na€_ve TNBC was collected (N ¼ 20).

Myeloid cell sorting, scRNA-seq, and library preparation

4

HLA-DRþCD11cþ myeloid cells from TUM- and JT-infiltrating cells were FACS sorted (ARIA-BD Biosciences). DAPI solution (BD Biosciences) used for dead cells exclusion. CD11c (BioLegend, catalog no. 301605, RRID:AB_314175), HLA-DR (BioLegend, catalog no. 307616, RRID:AB_493588), CD45 (BD Biosciences, catalog no. 557833, RRID:AB_396891), CD3-CD56-CD19 (BioLegend, catalog no. 300424, RRID:AB_493741; BD Biosciences, catalog no. 557919, RRID:AB_396940; BD Biosciences, catalog no. 557921, RRID: AB_396942) were included for the staining. Single-cell suspension was loaded into a Chromium Single Cell Chip (10× Genomics) according to the manufacturer's instructions. Target capture rate correspond to 5,000 to 10,000 individual cells/sample. scRNA-seq libraries were prepared using Chromium Single

4 Cell 30 v3 Reagent Kit (10X Genomics, catalog no. CG000183) according to manufacturer's protocol. Indexed libraries were

5 equimolarly pooled and sequenced on an Illumina NovaSeq 6000 system using paired-end 28 _ 91 bp as sequencing mode. A coverage corresponding to 50,000 reads/cell was obtained. For further details see the Supplemental material.

CAF primary cell lines, culture with monocytes, and migration assay

FAPþCD29þ and FAP–CD29þ CAF primary cell lines were established based on previously described protocols (7, 19, 22). For further details about migration assay see the Supplemental material.

6 Establishment of CAF primary cell lines and culture with monocytes

Both FAPþCD29þ and FAP–CD29þ CAF primary cell lines (from maximum 6–9 culture passages) were seeded in DMEM (10% FBS and 1% Pen-Strep) in 6-multiwell at the concentration of 2 _ 104 per well; complete DMEM (Hyclone, catalog no. SH30243.01) was replaced at 24 hours and 5 _ 105 CD14þ monocytes enriched by Miltenyi kit (Miltenyi, catalog no. 130–050–201; from HD and TNBC) were added. Both FAPþCD29þ and FAP–CD29þ CAFs plus CD14þ monocytes were incubated 3 days at 37_C. Each experiment was performed three times, including four HD and four TNBC patients, in

7 triplicates, for a total of 12 HD and 12 TNBC pts. Freshly isolated (day 0) and day 3 monocytes were evaluated by FC for both cocultures. FAP–CD29þ CAF cocultures were performed twice. FAPþCD29þ were seeded as described above, after ON CAFsupernatants were collected and freeze at −20_C.

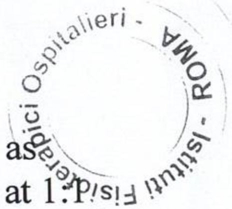
Miltenyi freshly isolated CD14þ monocytes from HD (N ¼ 3) and TNBC (N ¼ 3) were cultured for 3 days with FAPþCD29þ supernatants. CAF supernatants were obtained from three different CAF cell lines. To assess the differentiation, FC analysis was performed at day 3.

8 Mono-derived LAM-T cell cocultures in vitro

Circulating CD14þ monocytes from HD and TNBC patients were coculture with FAPþCD29þ and FAP–CD29þ CAF as described above. Upon 3 days of monocyte-CAF cocultures, mono-derived LAMs were recovered and then cocultured at 1:1 ratio with autologous CD3þ T cells (Pan T-cell isolation kit, Miltenyi, catalog no. 130–095–130) from either peripheral blood mononuclear cell (PBMC) of HD or patients with TNBC. CD3þ T cells were prelabeled with carboxyfluorescein diacetate succinimidyl ester (CFSE; 5

9 mmol/L; Invitrogen) for 15 minutes at 37_C. 96 plates were precoated with soluble anti-CD3 (1mg/mL; Clone OKT3, Thermo Fischer Scientific, catalog no. 16–0037–81) in PBS1x, 2 hours at 37_C. Mono-derived LAM and CD3þ T cells were cocultured in complete RPMI medium (Gibco, catalog no. 61870036; 10% FBS and 1% Pen-Strep) for 3 days, plus IL2 (100 U/mL). After 3 days, T cells were assessed for CFSE dilution, PD1, and CD25 expression. Three independent experiments were performed.

Animals



Istituti Fisioterapici Ospitalieri - ROMA

9

Mice used in this study include wild-type (WT) C57BL/6J and Trem2–/– animals bred at Washington University School of

10

Medicine (St. Louis, MO) animal facility. All animals were backcrossed until at least more than 98% C57BL/6J confirmed by genotype-wide microsatellite typing. Mice were housed under specific pathogen-free conditions, were all females and age matched. Mice from different genotypes (Trem2þ/þ and Trem2–/–) were cohoused from birth and separated during the experiment (the day of tumor injection). Mice did not undergo any procedures prior to their stated use and were injected at 8 to 10 weeks of age. All studies performed on mice were done in accordance with the Institutional Animal Care and Use Committee (IACUC) at Washington University in St. Louis. IACUC at Washington University in St. Louis have approved these studies.

11

Tumor models

PY8119 cells (purchased from ATCC, CRL-3278) were washed and resuspended in PBS. 5_105 cells were injected into the mammary fat pad (intramammary) that was previously shaved. Mice were monitored every day and tumors were measured by caliper every other day. Mice were sacrificed at day 15 and or day 30. For FC in human and mouse, see Supplemental material.

Statistics

Wilcoxon matched-pairs test, two-tailed; paired matched t test, two tailed; unpaired t test, two-tailed, were applied to compare

12

groups of FC analysis, levels of cytokines/chemokines by Luminex and in vitro CAF-monocytes cocultures. Two-way ANOVA test (Tukey multiple comparison test) performed for the migration assays in vitro. Mann–Whitney test was used for mice experiments. $P < 0.05$ was considered statistically significant in all tests. Correlations were calculated using the nonparametric Spearman and/or Pearson correlation test, two-tailed. Mouse data were shown as mean _ SEM. Two-way ANOVA for repeated measures was used to model longitudinal tumor growth between groups followed by posthoc comparisons on group difference

13

measures was used to model longitudinal tumor growth between groups followed by posthoc comparisons on group difference at time points. Mann–WhitneyUtest was used to compare two groups. Statistics were calculated with GraphPad Prism 6 and 8 (GraphPad Software).

Data availability

Data generated from this study are publicly available in Gene Expression Omnibus (GEO) repository: GSE206637 (bulk data) and GSE206638 (single-cell data).

Domande Informatica

1. Cos'è word
2. Cos'è il backup
3. Definizione di motore di ricerca
4. Definizione di software
5. Definizione di scanner
6. Che cos'è Excel
7. Cosa è la firma digitale
8. Cosa è la PEC?
9. Cos'è un data-base?
10. A cosa serve il programma Power Point?
11. Cosa sono: Chrome, Mozilla, Opera, Edge, Safari ?
12. Cosa è un firewall?
13. Cosa è un antivirus?