The Metastatic Cascade

Kitamura, Qian and Pollard Nature Reviews: Immunology 2015
The Tumor Microenvironment

Joyce and Pollard 2009
Wynn, Chawla and Pollard Nature 2013
CSF-1 Regulated Macrophages Promote Tumor Progression and Malignancy

Primary Mammary Tumor

- With CSF-1
- - CSF-1
  - -CSF-1 +CSF-1 Tg

F4/80 Staining

Lin et al 2001 J.Exp. Med
CSF-1 Regulated Macrophages Promote Tumor Progression and Malignancy

Primary Mammary Tumor

With CSF-1

- CSF-1

- CSF-1 + CSF-1 Tg

op/op + CSF-1 Tg

op/op

+/op

F4/80 Staining

Delayed Tumor Progression
No Pulmonary Metastasis

Aggressive Tumors
Pulmonary Metastasis

Lin et al 2001 J.Exp. Med
CSF-1 Regulated Macrophages Promote Tumor Progression and Malignancy

Primary Mammary Tumor

With CSF-1

- CSF-1

- CSF-1 + CSF-1 Tg

F4/80 Staining

Over-express CSF-1
More aggressive
Increased Metastasis

Delayed Tumor Progression
No Pulmonary Metastasis

Aggressive Tumors
Pulmonary Metastasis

Lin et al 2001 J.Exp. Med
Macrophage Diversity Promotes Tumor Progression and Metastasis

Activated Macrophage
*IL-12, MHC II^hi iNOS+, TNFa+, CD80/86*

Invasive Macrophage*
Wnt Signaling, CTS B&S+, EGF+

Perivascular Macrophage
Phagocytic

Macrophage Phenotypes: CD11b+
F4/80+ CSF1R+ GR1-

Inflammation

Immune regulation

Intravasation

Tumor cell invasion

Angiogenesis

Seeding at distant sites

Metastasis Associated Macrophages*
VEGFR1+, CCR2+ CXCR4- Tie2-

Angiogenic Macrophage*
VEGFR1+, VEGF+, Tie2+, CXCR4+
CTS B&S+

Immunosuppressive Macrophage
Arginase+, MARCO+, IL-10+, CCL-22+

Qian and Pollard Cell 2010
Tumor Cell Escape is Promoted by Macrophages

Kitamura, Qian, Pollard NRI 2015

Nature Reviews | Immunology
Survival of Women with Breast Cancer has dramatically improved.
Breast Cancer Survival after distant Recurrence by year of Recurrence

DRFI \leq 3\ year (P=0.13)

DRFI > 3\ year (P=0.45)
Angiogenic dormancy

Micrometastasis

Macrometastasis

Invasive cancer cell
Platelet aggregate
Endothelial cell
Immature BMDC
Dormant tumor cell
Quiescent stromal cell

Apoptotic/necrotic tumor cell
Lymphocyte
Pericyte
Mature BMDC
Blood vessel
Activated stromal cell

Macrophages and Metastasis
Experimental metastasis assay and quantification using stereological methods

Metastasis volume is estimated using: $V(\text{obj}) = t \times \sum a(\text{obj})$
**CSF1**<sup>op</sup> Null Allelic Frequency Determines Metastatic Capacity

Colony stimulating factor 1 - major growth factor for macrophages--- **Csf1**<sup>op</sup>
A Uniquely Defined Macrophage Population is Recruited

The Same Population is Recruited to Spontaneous Metastasis
Depletion of CD11b+ Macrophages Inhibits Metastatic Seeding and Growth
Tumor cell proliferation and extravasation is correlated with macrophage interaction in lung

48 hr

72 hr

GFP macrophage
CFP tumor cell
CD31-AF647 vessel
Extravasation, Survival and initial proliferation of tumor cells in the lung is affected by macrophage depletion

Doubling Time 16 vs 34 hr
CSF-1 Regulated Macrophages Promote Tumor Cell Viability

Qian et al. 2009
MAMS Promote Tumor Cell Viability through VCAM-1 Signaling

Qian ... Pollard, JW Plos One 2009, Nature 2011
Chen Q, Zhang XH, Massague J Cancer Cell 2012
At least 3 Mononuclear Phagocytic Systems

Schultz ... Pollard ... Geissmann Science 2013: Merad, Ginoux et al, Jung et al
Lung metastases preferentially recruit inflammatory monocytes

Bone marrow \[\rightarrow\] FACS

<table>
<thead>
<tr>
<th>Inflammatory Mono (IM)</th>
<th>Resident Mono (RM)</th>
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<tbody>
<tr>
<td>Ly6C+</td>
<td>Ly6C−</td>
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</table>

\[\rightarrow\] Blood

Primary tumor \[\rightarrow\] Lung metastasis

18hr

Expression of CC-chemokine receptor **CCR2** is much higher in IMs than RMs


**Chemokine mediated recruitment?**

**Contribution of CCL2-CCR2 signaling to the recruitment?**

Qian, B.Z. *et al.* *Nature* (2011) **475**: 222-
CCL2 is required for IM recruitment and following MAM accumulation

Met-1 → IM + a-CCL2 Ab → Lung
7hr

IM → cCCL2 Ab → Lung
18hr

Met-1 + a-CCL2 Ab → Lung
36hr

Ex vivo imaging

MacGreen

Cancer cells → Macrophages → Endothelial cells

% of recruited IMs in CD45+ cells

Qian et al. Nature (2011)

No. of macrophages / tumor-cell interactions

IgG vs aCCL2

Qian et al. Nature (2011)
Anti-CCL2 Antibody Inhibits Macrophage-Tumor Cell Interaction and Extravasation in vivo

Qian et al. Nature 2011
Tumor Derived CCL2 is Required for Metastasis

- Fold Vs GAPDH
  - control RNAi
  - CCL2 RNAi

- Mets Index (total Mets volume / Lung volume)
  - Ctrl RNAi
  - CCL2 RNAi

- Cell Number vs Time
  - 0hr, 24hr, 36hr, 48hr

- Primary Tumor
- Metastatic Tumor
Tumor and Stromal CCL2 is Required for Human Metastasis

MDA-231 spontaneous metastasis

MDA-231 Experimental Metastasis/
Treated 3 days after seeding

Day 22 Lung Alu / B-Actin

Relative Mets burden

4173 Experimental Metastasis

CCL2 blockade

Qian et al. Nature 2011
Neutralizing Anti-CCL2 Antibody blocks Tumor Cell Extravasation

Qian et al. Nature 2011
Neutralizing Anti-CCL2 Antibody blocks Tumor Cell Extravasation

Qian et al. Nature 2011
Conditional Ablation of VEGF in Monocytes

$Csf1r$-iCre$^{ER}$, $Vegf^{F/F}$ $X$ $Vegf^{F/F}$

Cancer cells

$Csf1r$-iCre$^{ER}$: $Vegf^{F/F}$ $-\text{Tamoxifen}$ $\downarrow$ $+\text{Tamoxifen}$

Qian et al. Nature 2011
Conditional Ablation of VEGF in Monocytes

Csf1r-iCreER: Vegf^F/F × Vegf^F/F

Cancer cells

Csf1r-iCreER: Vegf^F/F

Csf1r-iCreER: Vegf^F/F

Qian et al Nature 2011
Monocyte Derived VEGF is Required For Metastatic Tumor Cell Seeding

FVB/N.Csf1r.Mer2icre;Vegf$^{floxed/floxed}$

Qian et al. Nature 2011
Monocyte Derived VEGF is Required For Metastatic Tumor Cell Seeding

FVB/N.Csf1r.Mer2icre;Vegf$^{floxed/floxed}$

Qian et al. Nature 2011
Macrophage Derived VEGF Stimulates Extravasation by Increasing Vascular Permeability

Qian et al / Nature 2011
Macrophages Promote Tumor Cell extravasation, Survival and Persistent Growth

Kitamura, Qian, Pollard NRI 2015