

Membrane-localized Keratin-14 promotes invasion

Yohanes Tsehay
& Andrei Kucharavy

Joel Bader's Lab

10/04/2018



Overview

- Objective
- Methods
- Results
- Discussion
- Future Directions

Quantifying the Invasive Potential of Tumors

- Why would we need to make a system that quantifies the invasive potential of tumors?
 - Quantitative phenotypes are more amenable to systemic analysis
 - They can be used in population genetics methods, where genetic variations and invasive potential of tumors can be studied by directly perturbing candidate genes
 - Such studies can lead to the discovery of new drugs for cancer therapy which can ultimately have a positive impact on the poor prognosis seen in patients with metastatic carcinoma

Phenotype quantification allows us to pull out the molecular correlates

- Invasiveness quantification allows us to calculate the genetic contribution of individual genes
 - Quantitative characterization allows us to detect even minor contributors
 - Quantification of phenotype allows us to calculate the interaction between different genes
 - The fine-grainness of the quantification allows us to perform genetic correlation at the genome scale

Why we use Keratin 14 as a proof of expression?

- Keratin 14 is a known biomolecular marker for metastasis
- [Shamir .. Bader Ewald 2014] challenge the epithelial-to-mesenchymal transition requirement for metastasis, and instead, provide evidence in support of a model based on Twist1 induced dissemination of cytokeratin positive epithelial cells
- [Cheung .. Bader Ewald 2016] demonstrated that tumor cells only can invade and metastasize in clusters
- in the same paper, Cheung et al. suggested Keratin 14-associated pathways are key regulators of metastasis

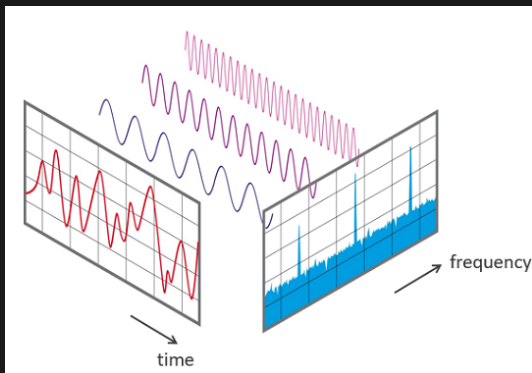
Project Objective

- We will test whether K14 is correlated with invasiveness:
 - 1 Total K14
 - 2 Peripheral K14
 - 3 Central K14

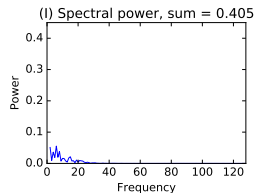
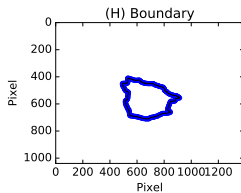
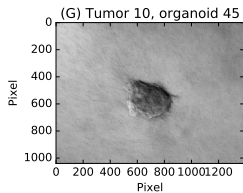
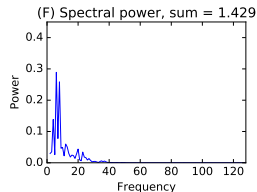
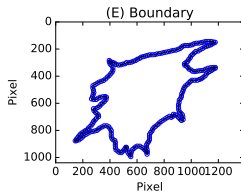
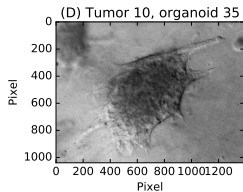
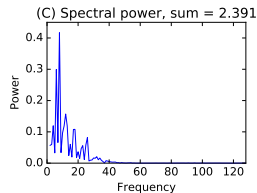
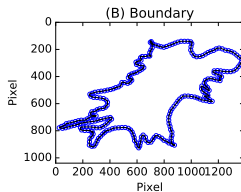
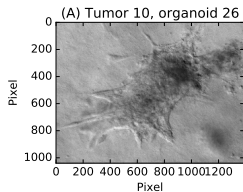
Our Data

- MMTV-PyMT transgenic mice were used to produce a supply of organoids
- Tumors were removed from 4 mice and a total of 90 - 150 small and large organoids were generated per tumor (\approx 300 organoids in total per tumor)
- The organoids were imaged using differential interference contrast (DIC) microscopy
- The images were manually traced using IMAGEJ to define organoid boundaries
- corresponding K14 images are available

Fourier Transform reminder



Example



Analysis

- Features that we considered to compare against our model include the following:
 - Fractional area of the organoid
 - K14 Mean
 - K14 Sum of pixels intensities in the periphery
 - K14 Sum of pixels intensities in the center
 - K14 total pixel intensity sum

An Efficient Way to Get Peripheral Pixels

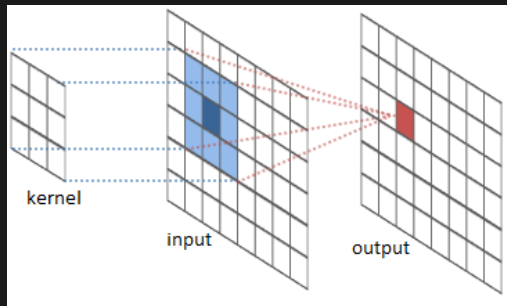
- 1 Generate a mask from the boundary
- 2 make a disk to be used as a kernel with which to convolve the mask

Example

A disk with a radius of 3 is:

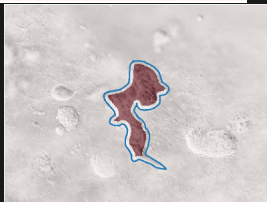
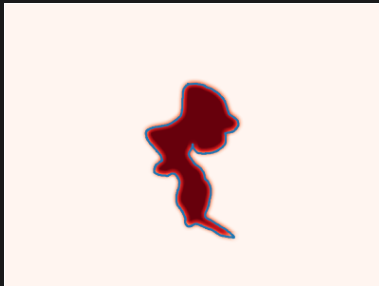
$$k = \begin{bmatrix} 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \end{bmatrix}$$

Convolution



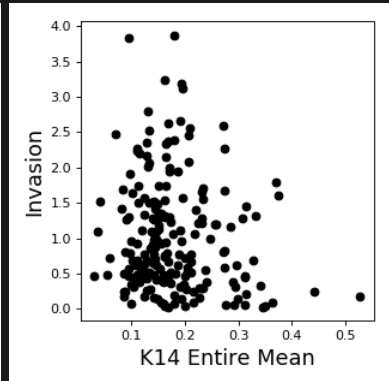
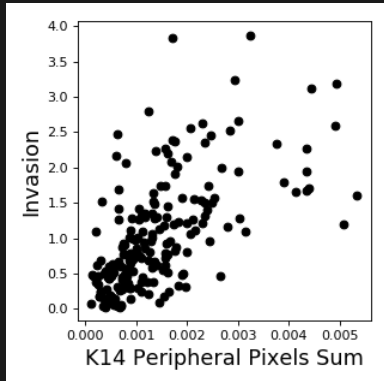
$$(f * g)[i, j] = \sum_{k=\min W_{kernel}}^{\max W_{kernel}} \sum_{l=\min H_{kernel}}^{\max H_{kernel}} f[i - k, j - l] g[k, l]$$

Convolution for efficient organoid segmentation



radius, $r = 20$ pixel size $\sim 0.5\mu$

Peripheral K14 expression correlates with invasive potential



Peripheral K14 explains correlates with invasive potential the best

	r^2 by mouse			
Organoids (PyMMT mice)	1	2	3	4
Peripheral sum K14	0.39	0.43	0.29	0.123
Central sum K14	0.21	0.19	0.11	0.010
Entire sum K14	0.22	0.22	0.12	0.013
Size	0.27	0.33	0.27	0.076

Peripheral K14 explains correlates with invasive potential the best

	r^2 by mouse			
Organoids (C1tag mice)	1	2	3	4
Peripheral sum K14	0.11	0.25	0.06	0.03
Central sum K14	0.01	0.03	0.06	0.00
Entire sum K14	0.01	0.00	0.01	0.00
Size	0.07	0.01	0.07	0.06

Discussion

- Looking at K14 expression in the periphery of the organoids is promising
- K14 expression in the center of the organoids correlates less with spectral score than peripheral K14 expression
- K14 Mean doesn't seem to contribute much
- Using peripheral K14 expression seems to have more significance for large organoids
- Fractional area and peripheral K14 expression give comparable r^2 values

Future Directions

- Apply our model on human organoids generated from breast tumors

Thank you!

- Joel Bader
- Joel Bader lab members
- Andrew Ewald
- Veena Padamanaban (data)
- Andrew Ewald members
- Cancer Target Discovery and Development