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Dissecting tumorigenesis and metastatic properties of cell lines by phenotypic functional assays and plasticity ratio (PR)

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Introduction

1. Our first poster (#2868) describes the creation of an in vitro platform of phenotypic assays that summatively represent metastatic biology and identifies Plasticity Ratio (PR; ratio of mesenchymal to epithelial markers) as a critical determinant of metastasis and other functional properties of a tumor cell population.
2. The relevance of epithelial to mesenchymal transition (EMT) and mesenchymal to epithelial transition (MET) have long been of interest and debate[1], but with the emerging concept of hybrid[2] or partial EMT[3] it is clear that the E-M axis determines and defines functional properties that might drive metastasis.
3. Therefore, one key strategy to target cancer metastasis would be to fix cells on this E-M axis[4], targeting plasticity and dormancy[5].
4. We hereby show that the plasticity ratio can summatively represent cells in the E-M axis and help differentiate between tumorigenic, metastatic, or dormancy properties.

Method

1. Metastatic biology was broken down into sixteen cell-based phenotypic assays. (AACR@2021, #2868)
2. Cells with high PR (greater number of mesenchymal to epithelial markers) were engineered and compared with the non-engineered wild-type cells with low PR (greater number of epithelial to mesenchymal markers) to create baseline data (Table 1). (AACR@2021, #2868)
3. Complex assay systems were created, to understand differential invasion (A), intravasation (B), chemoresistance (C), and secondary cross talk (D) a few examples of which are represented in Figure 1.

Results

Table 1

Name	HT29	HCT # 10GB7	HT #12BC6	HT #8C5	Colo 205	HCT 116	SW 480	SW #1C3
Engineered	No	Yes	Yes	Yes	No	No	No	Yes

Increasing PR

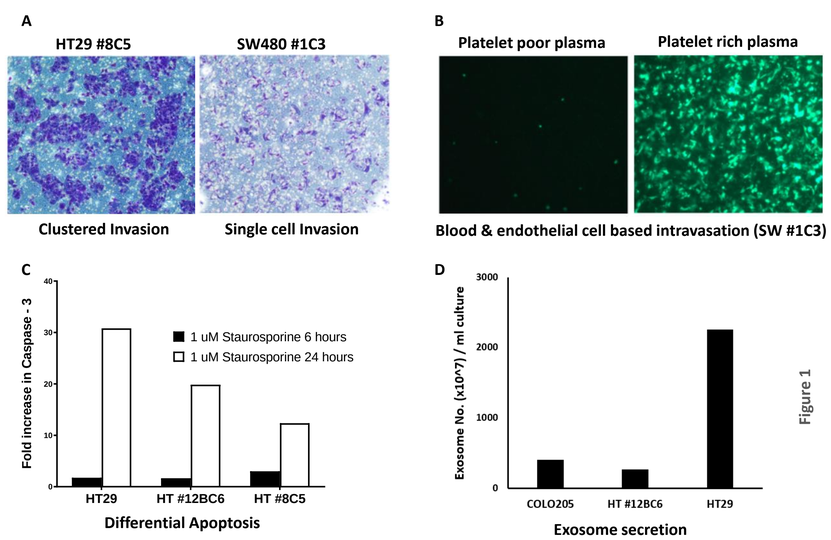


Figure 1

Translation of assays from 2-Dim to 3-Dim (Fig 2A); e.g., EMT (2B, 2C) invasion (2D).

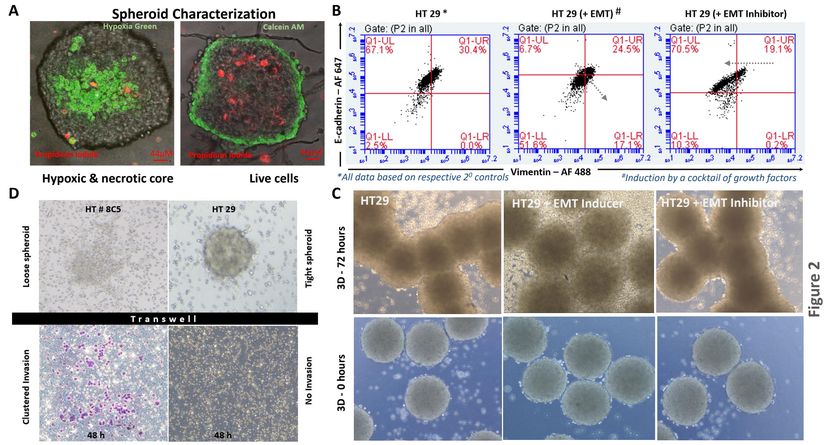


Figure 2

PR shows significant correlation with the metastasis marker, Snail.

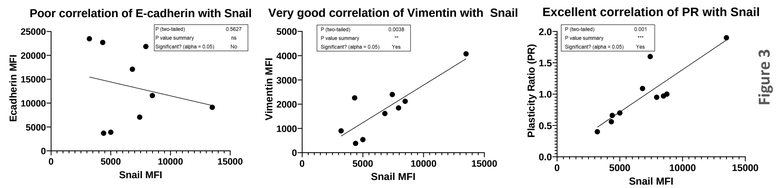


Figure 3

PR correlates with increased invasion & chemoresistance.

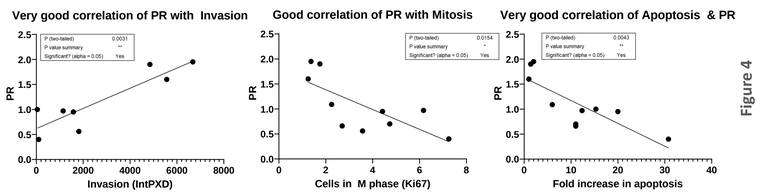


Figure 4

PR correlates with dormancy.

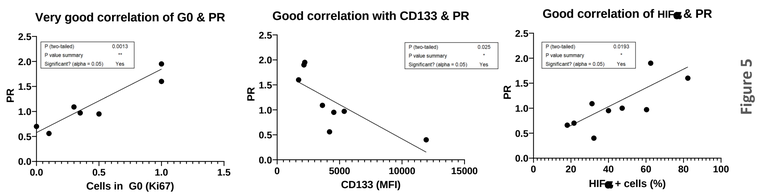


Figure 5

PR correlates with metabolic shift and autophagy

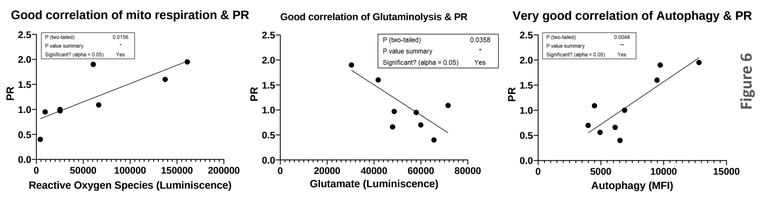
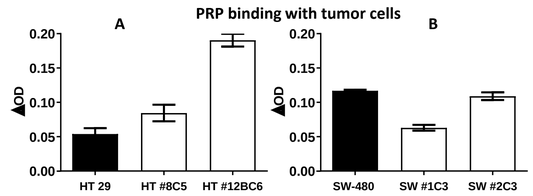


Figure 6

Effect of PR increase is minimal in SW 480 clones, suggesting saturating threshold



- PR doubles for HT29, with a concomitant increase in PRP binding (A)
- Minimal increase in SW 480 PR, with no increase in PRP binding (B)

Figure 7

CRISPR / CAS 9 engineered HCT 116 with decreased PR is functionally different

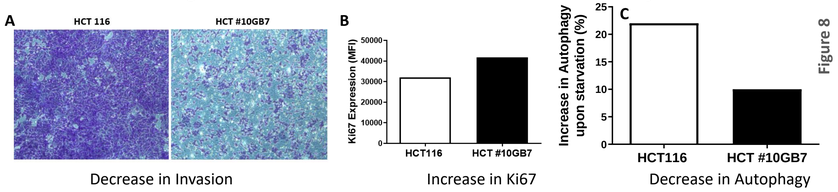


Figure 8

In Vivo PoC: Tumorigenesis depends on PR

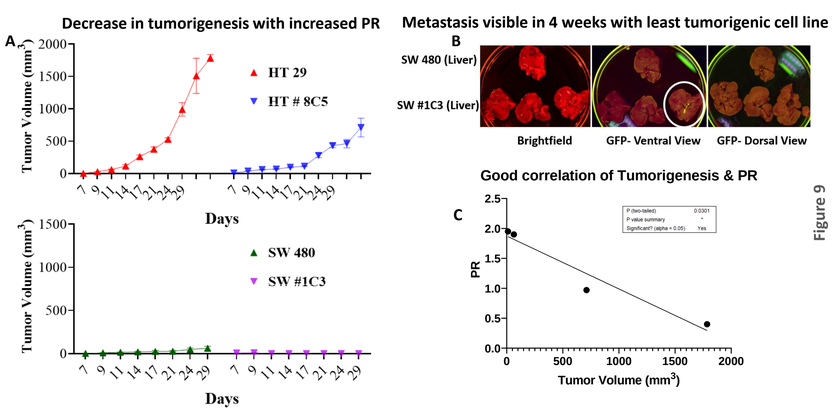


Figure 9

Summary

- ✓ Plasticity Ratio (PR) can successfully differentiate between multiple steps in the metastatic cycle, including tumorigenesis, invasiveness, dormancy, and surviving ability in the secondary tissue.
- ✓ Initial proof-of-concept in vivo experimentation also suggests that high tumorigenesis need not translate into successful metastasis.

Way Forward

- ✓ Normalize PR data with patient sample analysis to facilitate translational studies.
- ✓ Identify key targets in relevant steps of metastasis for targeted discovery leveraging the proprietary platform METSCAN™.

References

1. *Trends in Cell Biology, 2020, 30, 764-776*
2. *Nature, 2018, 556, 463-468*
3. *Cell, 2016, 166, 21-45*
4. *Nat Rev Cancer, 2019, 12, 716-732*
5. *Nat Med, 2021, 27, 34-44.*

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