A meta-metastasis analysis identifies pan-cancer markers and therapeutic targets

B027

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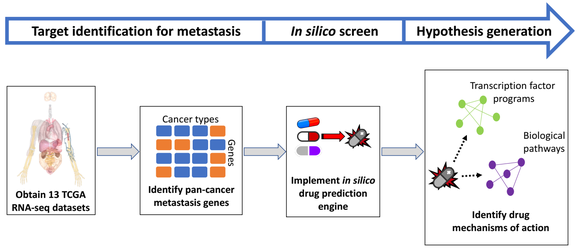
Introduction

Metastasis is a leading cause of cancer-associated deaths across several cancer types, yet the molecular details of its development have not been fully elucidated. Transcriptomic analysis can provide insight into the gene expression changes in the tumor that confer metastatic potential even during early stages of tumorigenesis. Here, by leveraging RNA-Seq data collected by The Cancer Genome Atlas (TCGA), we systematically identified mechanisms consistently associated with metastasis in primary tumors from 4844 patients across 13 different cancer types via comparison of primary tumors from patients with primary site, node-negative disease with no recorded distant metastasis (N0 not M1) compared to primary tumors from patients with node-positive disease (N1, N2, or N3). Differentially expressed genes were first determined within each cancer type, to reduce tissue- or disease-specific effects. Subsequently, via meta-analysis, we combine these results to identify commonality across 13 different types of cancer. Next, using a proprietary drug prediction algorithm called Drugfinder, we identified drug candidates that can target metastasis across all cancer types and within specific cancer types.

Methods

Differential expression analysis and meta-analysis. From TCGA RNA-Seq, we identified genes differentially expressed between N123 and N0 patients for 13 cancer types using LIMMA (1, 2). Plate ID was used as a covariate to remove batch effects. Pan-cancer gene results were aggregated using the inverse variance weighting method to generate a meta-metastasis gene expression profile (3). We filtered for genes present in at least 2 datasets, and used the top 500 by p-value.



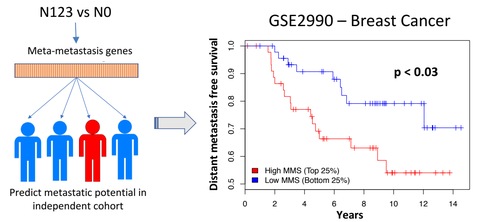
Survival analysis. Survival rates between top vs bottom quartile patient groups were compared via log-rank test (4).

Assessment of meta-metastasis profile. To evaluate if the meta-metastasis profile captures genes that inform metastasis progression in primary tumors, we interrogated metastatic potential of patients in an an independent dataset. This was quantified by calculating Euclidean distance between individual patient samples and the meta-metastasis profile. Survival rates were compared between the top and bottom patient quartiles.

Systematic In silico screening of anti-metastasis drug candidates. A drug signature database was assembled by combining data generated as part of the LINCS and CMAP projects. For each concentration, timepoint, and database combination, signatures were generated using LIMMA. Metastasis-associated gene results from the pan-cancer analyses were queried against the drug signature database using cosine similarity of the moderated t-statistic to assess gene expression reversal to identify novel drug candidates that reverse metastasis-associated gene expression.

Results

Meta-metastasis profile captures metastasis-associated genes and drives identification of novel therapeutic candidates

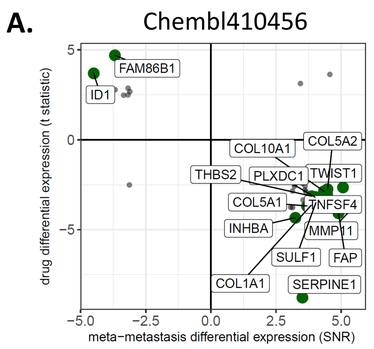


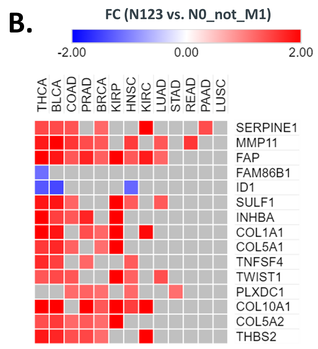
Meta-metastasis genes predict distant metastasis free survival in a independent cohort. Meta-metastasis gene expression profile was applied to an independent breast cancer cohort (n = 189) to assign a metastasis score. Patients with high predicted metastatic potential exhibited a significant increase in distant metastasis risk (log-rank *p < 0.03*).

Computational screening of anti-metastasis drugs yields 4 top candidates. Our drug discovery engine prioritizes compounds that reverse metastasis at specific concentrations and time points. This analysis also identified key components involved in reversal.

Drug_name	Conc (μM)	Time (hrs)	Similarity	adj p val	Occurrences by drug in database (all conc / times)	Occurrences by drug in top 5% of results	Target(s)
chembl410456	1	24	-0.0671	1.71E-08	8	6	CSNK1A1, CSNK1D, TGFBR1, MAPK14, PKD1
chembl3187109	10	6	-0.0531	7.45E-05	2	1	NA
chembl401570	3	24	-0.0527	7.67E-05	14	7	TGFBR1, MTOR
chembl237352	10	24	-0.0526	7.67E-05	6	4	TEK

Top overall drug candidate points towards importance of modulating the TGFβ and Wnt/beta-catenin pathways to suppress metastasis





(A) Scatter plot showing differential expression of key genes induced by Chembl410456 (1 μM, 24 hrs), and associated with metastasis. Labeled genes are the top 15 overall contributors to the reversal signature of the drug.

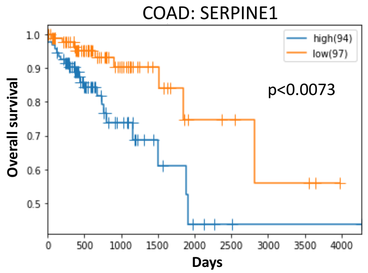
(B) Heatmap of the top 15 overlap between Chembl410456 (1 μM, 24 hrs) induced genes and top 500 meta analysis genes. Only the top genes affected by Chembl410456 are listed. Depicted are the fold changes between N123 and N0_not_M1 primary tumors for each cancer analyzed if nominally significant (Note: If |FC|>2, it has the max color)

Chembl410456 is the highest-ranked drug for ability to reverse the meta-metastasis gene profile. 6 of the 8 occurrences (concentrations/time points) of this drug rank highly indicating robustness across parameters.

Key targets of Chembl410456 include CK1 and TGFBR1. Chembl410456 (D4476) has been reported as a potent Casein Kinase 1 (CK1) inhibitor (5). TGFBR1 is one of the known targets of the drug listed in the Chembl database. Further, Chembl401570 also targets TGFBR1, suggesting that other drugs targeting the TGFβ pathway (6, 7) may help inhibit metastasis, including: galunisertib/LY2157299 targeting ALK5 (Eli Lilly), fresolumimab/GC1008 (Genzyme), and PF-03446962 targeting ALK1 (Pfizer).

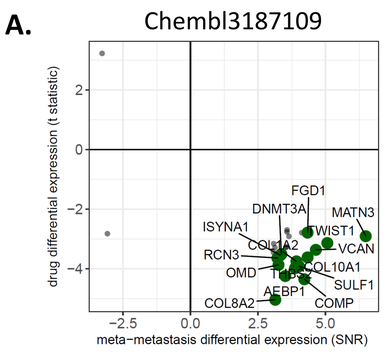
Chembl410456 simultaneously modulates both the Wnt (via CK1) and the TGFβ signaling pathways. These two pathways have been previously reported to interact as part of the epithelial-mesenchymal transition (EMT) (8), a key process driving metastasis (9). The top reversal genes were significantly enriched for a number of transcription factors including FOXC2, also linked to EMT (10). Noteworthy, one inconsistent observation is that suppressing CK1a has been reported to induce, rather than inhibit, melanoma-associated metastases (11).

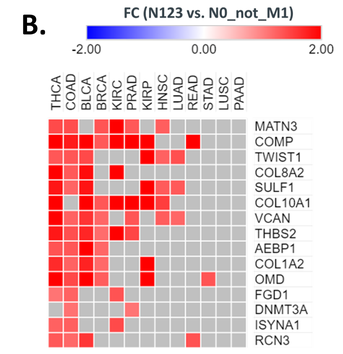
TGFβ inhibition may suppress metastasis via downregulation of SERPINE1

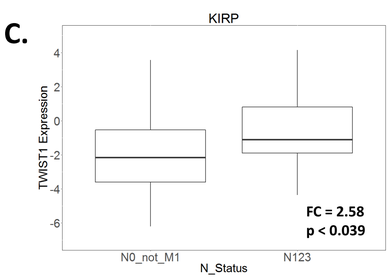


SERPINE1 was reversed most by Chembl410456. This gene is significantly associated with survival across a number of cancers including Colon Adenocarcinoma. It is known as plasminogen activator inhibitor 1 (PAI-1) and is associated with cell motility, which is essential in metastasis (12). Antibodies to PAI-1 block metastasis (13). SERPINE1 is part of the subset of EMT-associated genes that are induced by TGFβ1 in human malignant keratinocytes (14).

TWIST1 is a potential therapeutic target for inhibiting metastasis







(A) Scatter plot showing differential expression of key genes induced by Chembl3187109 (10 μM / 6 hrs), and associated with metastasis. Labeled genes are the top 15 overall contributors to the reversal signature of the drug.

(B) Heatmap of the top 15 overlap between Chembl3187109 (10 μM / 6 hrs) induced genes and top 500 meta analysis genes. Only the top genes affected by Chembl3187109 are listed. Depicted are the fold changes between N123 and N0_not_M1 primary tumors for each cancer analyzed if nominally significant (Note: If |FC|>2, it has the max color)

(C) Boxplot of TWIST1 expression in Cervical Kidney renal papillary cell carcinoma (KIRP), the cancer type in which the maximal upregulation in TWIST1 was observed.

Chembl3187109 (10 μM / 6 hrs) reverses meta-metastasis gene profile predominantly through TWIST1, COMP, and MATN3. COMP has previously been observed to be involved in metastasis (15).

TWIST1 is upregulated in primary tumors with lymph node involvement in 6 cancers and is directly downregulated by Chembl3187109 . TWIST1 has been shown to activate ADAM12 resulting in the formation of invadopodia and disruption of focal adhesions, thereby promoting cellular invasiveness and metastasis (16). The high ranking for Chembl3187109 also supports that other new or existing drugs targeting TWIST1 may inhibit metastasis, such as those described in (17).

Conclusions

- **Metastasis-associated genes** were identified by investigating 13 cancers in TCGA. This list can serve as a starting point to further the understanding of metastasis biology, assess the risk of a tumor metastasizing, and identify relevant drugs and targets.
- **Drugs reversing metastasis-associated genes suggest that metastasis may be suppressed by**
  - TGFβ pathway inhibition
  - Wnt/beta-catenin pathway modulation by CK1 inhibition
  - SERPINE1 downregulation (including by TGFβ pathway inhibition)
  - TWIST1 inhibition
- **These targets warrant further consideration alone or in combination to inhibit metastasis.**

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