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Signatures of Breast Cancer Metastasis at a Glance

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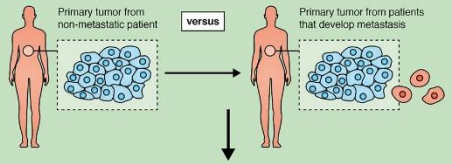
Biomarkers of breast cancer metastasis derived from gene expression profiling

NKI 70-gene signature (MammaPrint)



MammaPrint, Oncotype DX and PAM-50

- Generated from whole-tumor tissue (dotted boxes) without fractionation of different cell populations
- Dominated by proliferation markers
- Predicts tumor growth at metastatic sites (macro-metastases) and not the potential of the primary tumor cells to disseminate
- Lacks mechanistic information on the properties of the disseminating and metastasizing tumor cell subpopulations

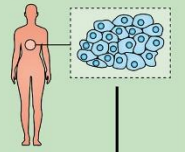
Oncotype DX

Review of literature for genes associated with outcome

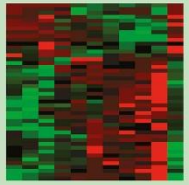
21 genes normalized to five reference genes

These include key factors involved in proliferation, invasion and estrogen signaling

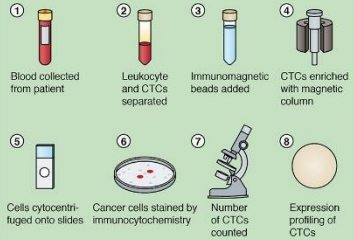
Molecular subtypes/PAM-50

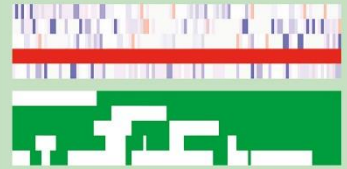


Hierarchical clustering of patient tumors based on only their gene expression



Molecular profiling of circulating tumor cells

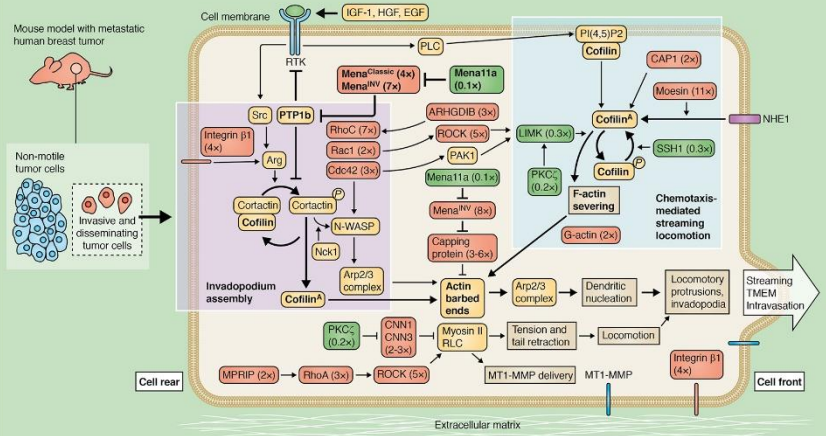




Circulating tumor cell signatures

- Provides mechanistic insights into breast cancer dissemination and metastasis
- Interpretation limited by leucocyte contamination during the CTC isolation process, tumor cell heterogeneity and origin of CTCs (e.g. not all CTC clones are capable of colonizing tissues; origin of CTCs is not certain)

HIS: motility pathways



Human invasion signature (HIS)

- Selective isolation of disseminating tumor cells from the primary tumor (dotted box) provides mechanistic insights into the metastatic process, and detailed molecular portraits of metastasizing cancer cells (shown within the 'cell' is the HIS motility/invasion pathway of disseminating tumor cells)
- Predicts metastatic recurrence, not growth potential

Key: Overexpression; Decreased expression

Part of the scheme in this panel has been adapted from a published figure (Patsialou et al., 2013, *Intravital*, 2, e25294).

Mena isoforms

Mena^{Classic}: no exon insertions; oligomerizes with other isoforms

Mena^{INV}: insertion of INV (invasive) exon; required for tumor cell invasion and dissemination

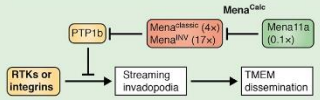
Mena11a: insertion of exon 11a; suppresses invasion and dissemination

Mena$^{INV-high}$: elevated expression (up to 17-fold) in disseminating tumor cells compared to non-disseminating tumor cells

Mena11a^{low}: decreased expression (10-fold) in disseminating tumor cells compared to non-disseminating tumor cells

Mena$^{INV-high}$ and Mena11a^{low}: isoform splicing pattern found in streaming tumor cells that use TMEM sites to intravasate and disseminate to distant sites; prognostic for metastasis

PanMena: antibodies that stain all Mena isoforms. Staining tissue with antibodies for PanMena and Mena11a yields Mena^{Calc} (Mena^{Calc} = PanMena – Mena11a).

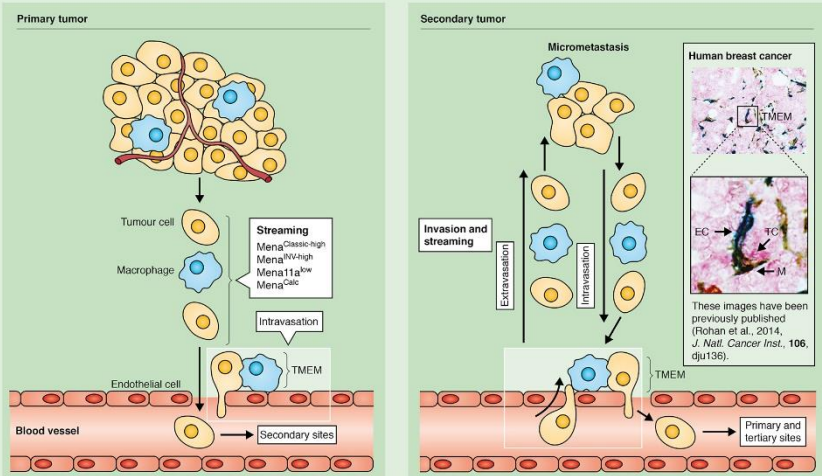


Clinical applications

Test	Assay	Platform	Risk stratification	Patient population
Oncotype DX* (Genomic Health), USA	21 genes FDA approved ASCO/NCCN guidelines	RT-PCR	Recurrence score <18 low, 18–30 intermediate, >30 high risk of recurrence within 10 years of diagnosis	Stage I or II ER$^+$, LN$^-$ and postmenopausal, ER$^+$, LN$^+$
MammaPrint (Agendia), USA	70 genes FDA approved	Microarray	Low risk (10%) and high risk (29%) of recurrence within 10 years of diagnosis	Stage I and II, ER$^+$ or ER$^-$, HER2$^+$ or HER2$^-$, LN$^-$ (US),
MapQuant Genomic Grade Index (Ipsogen), Europe	97 genes	Microarray	Low- and high-molecular grade	ER$^+$, intermediate grade tumors
Prosigna (NanoString), USA	58 genes (PAM50) + clinical variables FDA approved	Microarray	ROR (risk of recurrence in years 5–10 post surgery and endocrine therapy) LN$^-$: <40 low, 41–60 intermediate, 61–100 high LN+: <40 low, 41–100 high	Stage I and II ER$^+$, LN$^-$, or Stage II, ER$^+$, LN1–3 postmenopausal treated with surgery and 5 years of endocrine therapy
EndoPredict (Sividon Diagnostics), Europe	12 genes	RT-PCR	Low and high risk for recurrence at 5 and 10 years after diagnosis	ER$^+$, HER2$^-$, LN0–3
MammoStrat (Clarient Diagnostic Services)	p53, NDRG1, CEACAM5, SLC7A5 and HTF9C	Immunohistochemistry	Risk index score (high, moderate, low): risk of distant recurrence within 5 years of diagnosis	Stage I and II, ER*
Breast Cancer Index (BioTheranostics), USA	HoxB13, IL17RB BUB1B, CENPA, NEK2, RACGAP1, RRM2	RT-PCR	Low and high risk of distant recurrence within 10 years of diagnosis and low and high likelihood for endocrine therapy benefit	ER$^+$, LN$^-$
IHC4	ER, PR, Her2 and Ki67 ASCO guidelines	Immunohistochemistry	As per algorithm (Cuzic *et al.*, 2011, *J. Clin. Oncol.* **29**, 4273-4278)	All
Metasite (Metastat)*, USA	HIS-associated density of intravasation sites (TMEM)	Multiplex immunohistochemistry	TMEM score <6 low, 7–22 intermediate, >23 high	Stages I–III, ER$^+$, HER2$^-$, LN$^+$ or LN$^-$
MenaCalc (Metastat), USA	HIS-associated proportion of dissemination-competent cells	Multiplex immunofluorescence	Low and high risk (lower three versus top quartile of immunofluorescence intensity)	Stages I–III, LN0

*Comparison of Oncotype DX with TMEM score and the potential value when used as combined prognostics is illustrated in the accompanying text figure.

Role of TMEM and Mena^{Calc} in tumor cell dissemination



These images have been previously published (Rohan et al., 2014, *J. Natl. Cancer Inst.*, **106**, dju136).

Abbreviations: Arg, Abelson tyrosine-protein kinase 2; ARHGDIB, Rho GDP-dissociation inhibitor 2; BUB1B, mitotic checkpoint serine/threonine-protein kinase BUB1β; CAP1, adenylyl cyclase-associated protein 1; CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; CENPA, centromere protein A; CNN, calponin; Cofilin^A, activated cofilin; CTC, circulating tumor cell; EGF, epidermal growth factor; ER, estrogen receptor; HER2, human epidermal growth factor receptor-2; HIS, human invasion signature; HGF, hepatocyte growth factor; HoxB13, Homeobox protein Hox-B13; HTF9C, Hpa II tiny fragments locus 9c protein; IGF-1, insulin growth factor-1; IL17BR, interleukin-17 receptor B; LIMK, LIM domain kinase 1; LN, lymph node; Mena, mammalian-enabled; MPRIP, myosin phosphatase Rho-interacting protein; MT1-MMP, membrane-type matrix metalloproteinase 1; Nck1, NCK adaptor protein 1; NDRG1, N-myc downstream-regulated gene 1 protein; NEK2, never in mitosis A-related kinase 2; NHE1, Na^+/H^+ exchanger 1; N-WASP, neural Wiskott-Aldrich syndrome protein; PAK1, p21-activated kinase 1; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; PKCζ, protein kinase Cζ; PLC, phospholipase C; PR, progesterone receptor; PTP1b, protein-tyrosine phosphatase 1B; RACGAP1, Rac GTPase-activating protein 1; RLC, regulatory light chain; ROCK, Rho-associated protein kinase 1; RRM2, ribonucleoside-diphosphate reductase subunit M2; RTK, receptor tyrosine kinase; SLC7A5, large neutral amino acids transporter small subunit 1; SSH1, protein phosphatase Slingshot homolog 1; TMEM, tumor microenvironment of metastasis.

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