Pathology of Breast Cancer Metastases: Insights into Tumour Biology

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Breast Cancer Metastasis

- Heterogeneity between primary and metastases for key biomarkers – need for repeat biopsy
- Mechanisms of metastasis
- Intratumoural heterogeneity and insights into tumour evolution
- Circulating tumour DNA as a means of addressing heterogeneity – alternative to biopsy for monitoring disease and detecting resistance
Primary vs Metastasis

- Historical assumption that biology of metastasis matched that of the primary tumour – management based on characteristics of primary tumour
- Increasing understanding of intratumoural diversity and ongoing independent evolution at different sites – de novo and acquired (therapy)
- Recognition of need to rebiopsy metastasis
  - confirm diagnosis (benign 3-9%, other 1°)
  - retest key biomarkers; ER, HER2
Primary vs Metastasis

• Which metastasis do you biopsy?

• Practical considerations
  - ease of access, risk of complications, patient discomfort, cost

• Technical considerations
  - core vs FNA
  - bone -> requires decalcification so receptor testing less reliable

• ER concordance: LN 90%, liver 75%, bone 58%
  (Kamby et al., BJC 1989:60:252-7)
Primary vs Metastasis

- Criscitiello et al., Br Ca Res 2014;16:205
- Discordance rates from retrospective studies:
  - ER – 20% (6-40%): 24% loss, 14% gain
  - PR – 33% (21-49%): 46% loss, 15% gain
  - HER2 – 8% (0-43%): 13% loss, 5% gain
- Technical issues versus tumour biology
- Variation in studies; methodology, local recurrence vs LN vs distant mets
Primary vs Metastasis

BRITS (Breast Recurrence In Tissues Study)

• Prospective multicentre UK study looking at changes in receptor status on biopsy of recurrence and whether this impacts on management
• 205 pts -> 137 paired samples (88 local, 49 distant)
• 9% benign disease on biopsy
• ER 10% discordance: 8% +ve -> -ve, 2% -ve -> +ve
• PR 25% discordance: 16% +ve -> -ve, 9% -ve -> +ve
• HER2 3% discordance: 1 +ve -> -ve, 3 –ve -> +ve
• Change in management in 17% of patients
• No relationship to time to recurrence, but majority late recurrences so bias to ER+/ HER2- disease
Primary vs Metastasis

DESTINY

• Prospective single centre Canadian study
• 121 pts – 80% repeat receptors on biopsy
• ER 16% discordance: equal proportions + -> - and - -> +
• PR 40% discordance: 74% loss, 8% gain
• HER2 10% discordance: 20% loss, 8% gain
• No change in triple negative tumours
• Change in management in 14% of patients
• No association with survival but power low
• Loss of PR associated with shorter time to progression on endocrine Rx

Primary vs Metastasis

- Meta-analysis of studies looking at HER2 concordance [Houssami et al., BCRT 2011;129:659-74]
- Overall discordance 5.5%
- Nodal metastasis only – 4% (2-7%)
- Distant metastases – 11.5% (7-19%)
- Synchronous – 4.5% (2.5-8%)
- Metachronous – 11% (7-16%)
- No evidence for systematic direction of discordance
- Primary tumours HER2 heterogeneity is rare (<1%)
Primary vs Metastasis

• What does change in receptor status mean for survival?
• Limited data but several studies suggest worse outcome in discordant patients especially if loss of receptor expression
Primary vs Metastasis

• ER

• ER+ -> ER- worse survival
  +ve -> -ve 13% response to Tamoxifen
  +ve -> +ve 74% response to Tamoxifen
  [Kuukasjarvi et al., JCO 1996;14:2584-9]

• ER +ve -> +ve and ER –ve -> +ve similar survival; worse outcome if recurrence ER-ve regardless of 1° status
  [Khasraw et al., Curr Onc Rep 2011;13:17-25]

• Increased frequency ESR1 mutations in metastases versus 1° tumours (19% vs 7%) [Criscitiello et al., Br Ca Res 2014;16:205]
Primary vs Metastasis

- 182 HER2 +ve patients - loss of HER2 expression in metastasis 24% of cases
- Significant association between discordance and adjuvant chemotherapy – 27% vs 10%
- No association between discordance and adjuvant Trastuzumab
- Discordant patients showed worse overall survival, both with and without Trastuzumab therapy

Autopsy studies

• Wu et al., Clin Ca Res 2008;14:1938-46.
• 10 patients with advanced disease – rapid biopsy at autopsy
• Sites – bone 8, liver 7, adrenal 7, lung 10, GI 2 (lobular)
• ER – 4 +ve in 1° and mets, 4 –ve in 1° and mets, 2 +ve in 1° and –ve in mets
• HER2 9/10 cases –ve 1° and mets; one case 2+ with variable low level amplification across metastases
• Hormone receptor status consistent across metastatic sites -> intrinsic property of tumour
Autopsy studies

- 197 patients with advanced disease over period 50 yrs
- Sites – lung/pleura 81%, bone 74%, liver 72%, LN 55%
- 30% ≤3 sites, 39% 3-5 sites, 32% ≥ 6 sites
- CNS 27% with bone mets, 9% without bone mets
- Young women – liver, adrenal, gynae tract
- No association with type, grade, ER, HER2, Rx
- ER – 56% concordance, majority +ve -> -ve; loss in lung, bone, liver, LN’s
- HER2 – 89% concordance
- aCGH – majority of copy number changes shared between 1° and mets – early evolutionary events
- 4 cases showed gains in met not identified in 1° - amplified cells identified on FISH so minor clone metastasising
Intrinsic subtypes

• Gene expression profiles in 1° and metastasis very similar -> intrinsic subtype maintained between sites
• Highest concordance in different areas of 1° (0.9)
• Slightly lower for paired tumour and mets (0.82)
• Similarity maintained in metachronous mets (0.72)
• Greatest variation in extracellular matrix signatures reflecting differences in microenvironment
• Correlation between intrinsic subtype and 1st site of relapse
  luminal (ER+ve) -> bone
  HER2/ TNBC -> liver/ lung/ brain
• Least differentiated tumours spread to lung and brain – correlation with stem cell and proliferation signatures

Harrell et al., BCRT 2012; 132:523-35.
Intrinsic subtypes

Harrell et al., BCRT 2012; 132:523-35.
Models of metastasis

Linear progression model – sequential accumulation of genetic changes over time
• risk of metastasis increases with time/ tumour size
• metastasis recapitulates primary tumour

Parallel progression model – dissemination of metastases at an early stage with divergent evolution
• direct seeding of all metastatic sites from primary
• can have multiple waves of metastatic dissemination
• accounts for heterogeneity
• site specific selection of genetic changes
Models of metastasis

- Is the capacity to metastasise an intrinsic property of the tumour?
- Metastasis is a complex multistep process
- Evidence that primary tumours already contain gene expression profiles predictive of metastasis and poor survival and this is an early event in tumour progression
- Rapid metastasis – primary has all the necessary genetic traits for metastasis; poorly differentiated tumours with greater genetic plasticity
- Long latency – additional events required in tumour cells and surrounding microenvironment
Models of metastasis

Models of metastasis

- Seed and soil hypothesis – subpopulations of cells have tissue specific gene expression profiles that mediate metastasis to specific sites
- Identification of specific bone and lung metastasis signatures – presence in primary tumour predictive of site of metastasis

Minn et al., Nature 2005;436:518-24
Kang et al., Cancer cell 2003;3:537-49
Tumour evolution

Tumour evolution: heterogeneity

• Next generation sequencing – sequence and copy number information. Simultaneously sequences multiple subpopulations within a tumour.
• Identification of tumour subclones derived from a common clonal precursor
• Small number of common founder mutations; greater number of mutations present at low frequencies
• Findings support branched evolution; variation in mutation status between 1° and mets.
• Common genetic events indicate common progenitor
• Private genetic events indicate genetic divergence with independent evolution
• Mets may arise from minor subclones within 1° tumour
Tumour evolution: heterogeneity

- Primary versus metastasis in clear cell renal carcinoma
- 120 mutations: 40 ubiquitous, 59 shared, 29 private mutations unique to one site – ongoing clonal evolution
- Branched evolution with separate branches for 1° and mets
- One biopsy maximum of 55% of mutations
- Same protein inactivated by different mutations at different sites – convergent evolution

Gerlinger et al., NEJM 2012:366;883-92
Tumour evolution: heterogeneity

- Branched evolution
- 67% of driver mutations show heterogeneous distribution
- Dominant clone in one area may be minor clone in another – reliability of single biopsy?
- Parallel evolutionary events involving functionally important genes/ pathways – convergent evolution

Gerlinger et al., Nature Genetics 2014: 46(3):225-33
Tumour evolution: heterogeneity

- Branched evolution
Breast Cancer

• Heterogeneity identifiable at morphological level
• 20 breast cancers divided into sectors
• 9 monogenomic – homogeneous
• 11 polygenomic – multiple subpopulations; segregated and mixed distributions
• Subpopulations have common breakpoints – clonal origin
• Many subpopulations differ by a small number of focal events – occurred late following initiation and expansion

Navin et al., Genome Res 2010;20:68-80
ER positive tumours

- ER +ve breast cancer – sequencing of 50 single nuclei
- Highly similar copy number alterations – monoclonal
- 17 clonal mutations; 22 subclonal mutations in rare cells
  

- 77 patients – comparison of major mutations in primary and metastasis
- PIK3CA – 90% concordance
- AKT1 – 99% concordance
- PTEN expression – 93% concordance
- Ki67 – proliferation rate similar
- 90 gene expression panel – high level of overall correlation

  Schleifman et al., PLOS One 2014;9(2):e88401.
ER positive tumours

• Shah et al., Nature 2009;461:809-13
• ER+ lobular carcinoma with metastasis 9 years later
• 30 mutations in the metastasis
• 5 prevalent in primary; 6 minor subclones in primary
• 19 de novo mutations not identified in the primary
• Significant evolution of mutational content
TNBC

- TNBC – sequencing of 50 single nuclei
- 2 distinct cell populations
- 374 clonal mutations
- 145 subclonal mutations forming 3 clusters
- Increased mutation rate compared with ER +ve tumour
- Copy number alteration – early change followed by stable clonal expansion
- Gene mutations – gradual evolution over time

TNBC

• Single cell sequencing of grade 3 TNBC and liver metastasis
• 3 advanced cell populations; highly clonal with complex gene rearrangements – shared genomic patterns and distinct changes
• Breakpoint pattern suggested subpopulations arose early in tumour evolution
• H – anatomically segregated
• AA and AB – intermixed
• No further evolution in metastasis
• Punctuated clonal evolution – subpopulations distinct from root with no intermediate branches

Navin et al., Nature 2011;472;90-4
TNBC

- TNBC – primary and brain metastasis
- 48/50 mutations at both sites
- 20 similar frequency at both sites, 26 enriched in met, 2 enriched in primary
- 2 de novo mutations in met
- 96% of copy number changes in primary found in met
- Only 80% of copy number changes in met found in 1° - broader regions involved with expansion of region and selection of adjacent events
- Wide range of mutation frequencies in 1° consistent with heterogeneity, narrower frequency in met

Ding et al., Nature 2010;464;999-1005.
TNBC

- TNBC – variation in degree of clonal heterogeneity between tumours
- Wide spectrum of genomic evolution at diagnosis
- Greatest heterogeneity in basal BC
- Key events include p53 mutation – present at high clonal frequency

Heterogeneity: what does it mean?

- Single biopsies may not reflect genetic diversity; dynamic process = spatial and temporal
- Chromosomal instability itself is a marker of poor outcome
- Significance for molecular targeted therapy – may be low frequency subclones that are source of resistance to therapy
- Need to identify common driver mutations that occur early in tumour evolution
ctDNA: Liquid biopsy

• Single biopsy only gives a snapshot of tumour biology—need multiple or repeat biopsies to reflect spatial and temporal heterogeneity
• ctDNA = circulating tumour DNA. Small fragments of DNA in plasma arising from the tumour
• ctDNA is shed from all sites of tumour – represents complete repertoire of mutations present across multiple sites
• Identifiable in plasma so potential for monitoring with serial blood tests:
  total tumour burden
  can monitor several mutations simultaneously – clonal response with resistant subclones
  can detect new mutations – resistance to therapy
ctDNA: Liquid biopsy

ctDNA: Liquid biopsy

- ctDNA detectable in over 75% of patients with advanced disease, however only present in up to 50% of patients with early breast cancer

ctDNA: Liquid biopsy

- Untargeted screening using next generation sequencing
- 66 year old with ER+/HER2- breast cancer with associated liver mets
- 15 mutations in primary: all in met + two new mutations
- Metastasis enriched for mutations present at low frequency in primary, including ESR1 mutation associated with anti-oestrogen resistance

ctDNA: Liquid biopsy

- All mutations detected in ctDNA
- Initial decrease in ctDNA following initiation of Rx
- 6/12 later levels increased – preceded progression on imaging

ctDNA: Liquid biopsy

- ctDNA – alternative approach is to screen primary for selected mutations common in breast cancer using TAmSeq or digital pCR
- 52 patients; 30 had suitable genomic changes
- Mutations detected in 97% of women and 82% of samples
- Increased ctDNA levels associated with worse prognosis
- Serial assays reflected radiological response to Rx; changes preceded imaging changes by an average of 5/12
- Improved sensitivity and dynamic range compared with Ca15-3

Dawson et al., NEJM 2013:6(224):1199-209.
ctDNA: Liquid biopsy

- Can follow several mutations simultaneously
- Some cases showed similar dynamic changes over time
- Some cases showed divergent patterns of response to therapy suggesting presence of resistant subclones

Dawson et al., NEJM 2013:6(224):1199-209.
ctDNA: Liquid biopsy

• Recent study in colorectal cancer monitoring patients treated with EGFR inhibitors
• 70 new mutations appeared following commencement of therapy
• 50% developed mutations in KRAS codon 12 known to confer resistance to anti-EGFR Rx
• Overall emergence of KRAS/ MAPK pathway mutations in 96% of patients who develop resistance
• Average number of mutations in each patient was 2.9 – different resistance mutations developing at different metastatic sites (convergent evolution)

ctDNA: Liquid biopsy

• Limited use in screening setting: potentially detects tumours below resolution of current imaging; many mutations found in tumours from multiple organs so? Location

• Huge potential for monitoring response to therapy in metastatic and neoadjuvant settings

• Overcomes some of the issues posed by intratumoural heterogeneity

• Can identify development of resistance with early changes in targeted therapy
Heterogeneity in Clinical trials

- Heterogeneity being incorporated into clinical trial design – ‘adaptive trials’
- TRACERx trial for lung cancer