Genomic profiling of breast cancer leptomeningeal metastasis (BCLM) reveals a divergent metastatic evolution and therapeutic targets

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Rationale

Leptomeningeal metastasis is a devastating development in breast cancer:
• overall survival remains 3–4 months despite current treatments
• clinical trials of novel therapies exclude patients with BCLM
• there is a lack of access to leptomeningeal metastatic material
• there are no robust pre-clinical models

Project Aims

1. Identify BCLM molecular drivers and determine metastatic evolution, through genomic evaluation
2. Develop pre-clinical BCLM models using patient-derived leptomeningeal material

Patients and methods

Whole exome sequencing:
• ≥ 10 ng DNA was used to prepare libraries using Agilent SureSelect XT Low Input reagents and methodology
• Sequencing was performed on Illumina NovaSeq 6000 to a target depth of 200X
• Bioinformatic analysis pipeline using GATK and Mutect 2 to identify somatic variants, and CNVM to identify copy number alterations

3D in vitro culture of CSF tumour cells:
• CSF cellular content was suspended in 5 µL Matrigel in droplets, overlaid with organoid media (supplied by OCaIO)

Table 1: Patient and sample characteristics

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<th>Patient</th>
<th>CSF DNA</th>
<th>Plaque cDNA</th>
<th>Laminar cDNA</th>
<th>Turnover cDNA</th>
<th>Metastatic cDNA</th>
<th>Unique to CSF</th>
<th>Unique to tumour</th>
<th>Unique to CSF and tumour</th>
<th>Unique to CSF and not tumour</th>
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Figure 1) Clinical samples were collected from 21 patients with confirmed BCLM

(a) BCLM mutational profiles are distinct to extra-cranial metastases, with 74% (median) of CSF cDNA somatic variants not shared with the concurrently sampled plasma cfDNA. (b) Phylogenetic trees reveal a divergent metastatic evolution of BCLM compared to extra-cranial metastases (plasma/other sites). (c) Recurrently mutated and potentially actionable (shaded blue) Cancer Genome Census (CGC) genes were identified. (d) CNV events occurred more frequently in CSF, common events were 1q, 8q gain and 8p, 17p loss.

Results – BCLM Genomics

Results – BCLM pre-clinical modelling

(a) CSF cells from 5 BCLM cases have been expanded in vitro by 3D culture, and show heterogeneous morphologies. (b) CSF-PDxs underwent lentiviral infection to label with fluorescent and luminescence reporter genes. (c) 3D in vitro therapeutic testing has revealed BCLM models to be mostly insensitive to methotrexate, the standard chemotherapy agent used intrathecally. (d) Labelled CSF-PDxs were used to establish in vivo BCLM models by intra-CSF and intra-cardiac injection routes.

Conclusions and future directions

• BCLM are genomically distinct to extra-cranial metastasis
• BCLM develops private genomic aberrations which may be amenable to therapeutic targeting
• Patient derived in vitro and in vivo BCLM models have been generated and will be used to functionally validate driver aberrations
• An integrative approach to understanding BCLM will discover novel therapeutic approaches

Contact

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• Culture media for patient derived organoids obtained by partnership with OCaIO.

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