



Identifying drivers of metastasis; towards a systematic approach

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Metastasis is incontrovertibly linked to poor cancer patient survival. Cancer treatments that inhibit metastasis are predicted to improve cancer patient outcomes by preventing cancer dissemination. However, to control metastasis in patients necessitates an understanding of the biological drivers of metastasis. Drivers of metastasis can be thought of as factors that promote tumour dissemination, required at least for a part of the metastatic process. Yet, presently specific molecular drivers of metastasis largely remain unidentified, and very little evidence for ubiquitous metastasis-specific driver gene mutations has been identified to date (1,2).

Metastasis is a complex process, and it is becoming clear that identifying metastasis drivers requires novel approaches (3). Systems biology incorporates modelling of complex biological systems at the molecular, cellular, tissue and organismal levels, and so might be a suitable approach to study metastasis (4).

Metastasis refers to the acquisition of new aggressive behaviours, or hallmark properties (5) of cancer cells during tumour growth, following the initial development of a primary tumour. Of the different cancer types, melanoma is particularly aggressive and has a high propensity to metastasize early in its growth, at a stage when relatively few cell divisions have occurred (6). Despite remarkable advances in new treatments for metastatic melanoma in the past few years, outcomes for melanoma patients remain relatively modest due to frequently little or no treatment response, or alternatively some degree of initial response followed by resistance to therapy and relapse, or commonly occurring adverse side-effects (7). Targeting specific metastasis drivers may provide a revolutionary approach to treating cancer, and could lead to major advances in

improving patient outcomes.

In the article by Agrawal *et al.* (8) the authors have used a systems biology approach to investigate glycosylation changes in clinical melanoma tissue samples from patients. The overall hypothesis was that altered glycosylation patterns would be a potential driver of metastasis and would contribute to aggressive behaviour in melanoma. To address this hypothesis, the authors compared differences in glycoprotein glycosylation between matched primary and metastatic tumour tissues, and found an increase in core fucosylation (addition of a fucose residue in α 1,6 linkage to the first GlcNAc of the oligosaccharide core) mediated by fucosyl transferase 8 (*FUT8*) in metastatic melanoma. Improved sensitivity in the screening technology using lectin microarrays for low amounts of fixed tissue starting material to detect altered glycosylation in a cohort of patient samples was a key technical advance, providing a possible starting point for this research in using clinical samples. The authors used matched primary and metastasis melanoma tissues from a total of 17 patients, i.e., 34 samples in total to carry out a lectin microarray screen. Primary melanomas are small in size (often less than 0.5 mm³), and most of the primary melanoma tissue specimen had already been used for diagnosis, leaving little tissue available for screening. Additionally, primary melanoma tissue is frequently formalin-fixed and paraffin-embedded (FFPE), meaning that although archival tissue may be available, it needs to be deparaffinized first, and extensive cross-linking could limit detection sensitivity.

The authors found that metastatic tumours exhibited higher levels of poly/multi-antennary-*N*-acetyl-lactosamine, α -2,6 sialic acids, and core fucose, and lower levels of

α -1,2 fucose structures than the corresponding primary melanoma samples. Having identified these differences in the glycomic profiles in the patient tissue samples, the authors next correlated their lectin microarray data with publicly available transcriptomic data from TCGA, focusing on glycosyltransferase genes that would be predicted to be involved in the synthesis of glycan epitopes detected by the lectin and antibody probes present in the lectin microarray. Among the observed glycan changes, the metastatic melanomas exhibited higher expression levels of the core fucosyltransferase, *FUT8*, associated with core fucose, than did primary melanomas. Following this, the authors then went on to assess the effects of silencing particular glyco genes (glycosyltransferases) on cell invasion, the latter being a key property of tumour cells required for metastasis. Gene-silencing screens, using small interfering RNA (siRNA) mini-screens, were carried out on two melanoma cell lines (SkMel147 and WM3211), in which the selection of specific glycosyltransferase candidates to be knocked down had previously been identified as being either “pro-metastatic” or “anti-metastatic”. For example, changes in fucosylation affect key pro-metastatic processes such as adhesion and immune recognition. As a functional assay of invasiveness, trans-well invasion assays were incorporated into the screening methodology for the two cell lines, and once again *FUT8* was identified as one of the candidate pro-invasion/pro-metastatic genes to be selected for subsequent studies. *FUT8* is a fucosyltransferase that promotes core fucosylation (which is one of the most common types of glycosylation involving the attachment of a fucose residue to N-glycans, O-glycans and glycolipids) of target glycoproteins.

Following the identification of *FUT8* as a candidate glyco gene for inducing invasive behaviour in melanoma cells, the authors then demonstrated that *FUT8* silencing decreased the extent of *in vivo* melanoma metastasis occurring in xenograft tumours, using two metastatic melanoma cell lines, 113/6-4L (4L) and SkMel147, both of which express endogenous *FUT8* at high levels. In addition, the authors showed that *FUT8* expression is required in established metastases of 4L cells for further metastatic dissemination, using *in vivo* assays in mice.

The authors went on to show that up-regulation of *FUT8* expression in metastatic melanoma was mainly due to transcriptional activation, and that the *FUT8* promoter was predominantly responsive to changes in transforming growth factor β (TGF- β)-induced factor homeobox 2 (TGIF2) protein levels. TGIF2 binding to the *FUT8* promoter was enriched in accordance with high levels of

promoter activity and *FUT8* expression.

One hundred and fourteen glycoproteins were identified as being commonly expressed in all three of the 4L, SkMel147 and MeWo cell lines, and in which core-fucosylation was deregulated together with high *FUT8* expression. Gene ontology enrichment analysis for cell migration and locomotion control mechanisms was used to identify neural cell adhesion molecule L1 (L1CAM) as being common to all three cell lines, along with another 12 glycosylated proteins, including integrins α 4, 6, and V, integrin B1, ADAM10, laminins β 1 (LAMB1) and γ 1 (LAMC1), pro-low-density lipoprotein receptor-related protein 1 (LRP1), cell surface glycoprotein MUC18 (MCAM), plexin-A1, plexin B2, and neuropilin 2 (NRP2). L1CAM was identified as a key core fucosylated glycoprotein in melanoma that mediated the pro-invasive effects of *FUT8*. High levels of glycosylation in L1CAM protein had already been known to regulate cell attachment, invasion and migration in cancer, including melanoma progression (9). Core fucosylation in L1CAM was found to be required for promoting plasmin cleavage of L1CAM.

Thus, to summarize, using a systems-based approach to study glycomic changes and corresponding enzymes associated with melanoma metastasis in patient samples, the authors identified molecular changes in metastatic tumours, including altered glycosylation and elevated *FUT8* expression. These changes were associated with upregulation of core fucosylation by *FUT8*, and importantly, extensive functional analysis of invasion and metastasis, both *in vitro* and *in vivo* using mouse models underpinned the authors' assertion that *FUT8*-mediated core fucosylation acts as a driver of melanoma metastasis, while its inhibition suppressed invasion and tumour dissemination. Increased *FUT8* expression in metastatic melanoma was transcriptionally mediated by TGIF2 binding. Adhesion molecule, L1CAM, was identified as a glycoprotein target of *FUT8*, and was enriched in cell migration proteins in metastatic melanomas, and core fucosylation was found to impact L1CAM cleavage and the ability of L1CAM to support melanoma invasion. Therefore, the authors overall suggest that *FUT8* may represent a new potential therapeutic target for melanoma metastasis.

Aside from glycosylation enzymes identified by Agrawal *et al.* (8), abnormalities have been identified in the expression of dozens of genes associated with melanoma aggressiveness, and a number of these genes have been suggested as potential metastasis drivers (see *Table 1*). Transcriptional differences between primary and metastatic melanomas could subsequently lead to downstream molecular

Table 1 Examples of several candidate transcriptionally-mediated metastasis driver or suppressor genes

Candidate transcription-mediated metastasis driver or suppressor protein/gene name	Tumor type	Proposed metastasis function; "Driver" [†] or "Suppressor" [‡]	Reference
Fucosyltransferase 8/ <i>FUT8</i>	Melanoma	Driver	Agrawal <i>et al.</i> (8)
Osteopontin (OPN)/ <i>CD44</i>	Bladder	Driver	Ahmed <i>et al.</i> (10)
Midkine/ <i>MDK</i>	Melanoma	Driver	Olmeda <i>et al.</i> (11)
Transforming growth factor beta receptor 2/ <i>TGFBR2</i>	Gastric cancer	Driver	Nadauld <i>et al.</i> (12)
Early B Cell Factor 3/ <i>EBF3</i>	Melanoma	Driver	Chatterjee <i>et al.</i> (13)
Signal transducer and activator of transcription 3/ <i>STAT3</i>	Prostate	Suppressor	Pencik <i>et al.</i> (14)
Gabaa receptor alpha 3/ <i>GABRA3</i>	Breast cancer	Driver	Gumireddy <i>et al.</i> (15)
Metastasis suppressor protein 1/ <i>MTSS1</i>	Melanoma	Driver	Mertz <i>et al.</i> (16)
LncRNA/ <i>MALAT1</i>	Breast cancer	Driver	Jadaliha <i>et al.</i> (17)
CCAAT enhancer binding protein delta/ <i>CEBPD</i>	Urothelial carcinoma	Driver	Wang <i>et al.</i> (18)
Rab GTPase activating protein/ <i>TBC1D16</i>	Melanoma	Driver	Vizoso <i>et al.</i> (19)

[†], "Driver": increased driver expression enhances metastasis; [‡]"Suppressor": increased suppressor expression suppresses metastasis.

changes, some of which could be therapeutically targeted to inhibit metastasis in advanced cancer patients. However, does systems biology provide a suitable approach to confidently identify novel metastasis drivers, and if so, how can this process be efficiently exploited? But firstly, what exactly is a "novel metastasis driver".

Carrying out systematic screening, using multiple "omic" platforms on matched primary and metastatic tumour samples, as well as combined functional analysis incorporating *in vitro* and *in vivo* studies, together with analysis of additional patient cohorts, and/or additional molecular characterization, is very likely to be the basis of future systems biology approaches, with the aim of identifying metastasis drivers. Nevertheless, despite present understanding of the term "driver mutation" in cancer, defining what is meant by a driver of metastasis is somewhat looser, especially since gene mutations, it would appear, constitute only a small part of the spectrum of possible driver molecular events in metastasis. To avoid potential confusion or unclear thinking in this field, it may be necessary to better define exactly what is meant by the term "driver" in the context of metastasis.

Recently, a potentially systematic approach outlined several criteria to define driver changes, which could be acquired in metastasis, and which would lead to changes in gene transcription in metastatic cancer cells (13,20). The driver changes debated involved the acquisition of driver epigenetic changes, but the same criteria would be

applicable to transcriptional changes; accordingly several suggestions including considerations, approaches and models for the study of such metastasis drivers were made (13,20). Molecular changes may be identified in, and shared between many different metastatic tissue samples, and may be demonstrated to result in metastasis-related functional changes in tumour cells. It was asserted that these changes are more likely to be important in the context of metastasis than non-shared and/or functionally unrelated changes (13,20), which is broadly similar in concept to the frequency-based approaches used to identify cancer driver mutations (1).

Alterations showing a consistent enhancement, or reduction in their effect between matched metastatic versus primary tumour paired tissue samples, together with functional links to aggressive behaviour, and which are observed in multiple different metastatic samples, would fulfil several criteria that might be expected of candidate drivers of metastasis. Studies such as the one by Agrawal *et al.* (8) pave the way towards the establishment of a consensus on what constitutes metastasis drivers able to enhance the metastatic fitness of tumor cells (21), and therefore potential new therapeutic targets.

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