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What hope for the future? GNAQ and Uveal Melanoma.

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Abstract

Uveal melanomas (UM) are aggressive ocular tumours that spread to the liver. They are characterised by alterations of chromosome 3 and 8 which are highly predictive of a poor prognosis. Unfortunately, being able to identify those patients with aggressive disease has not, as yet, translated into improved survival. Recently mutations of Guanine nucleotide-binding protein G(q) subunit alpha (*GNAQ*, or G-alpha-q), that effectively turn it into a dominantly acting oncogene, have been identified in approximately half of UM. These mutations are specific to UM and other non-cutaneous melanomas and are not found in normal tissues, making them potential therapeutic targets. Here we review the background to *GNAQ* in UM and explore what makes it such an interesting target for the future treatment of patients.

Introduction:

Treatment for cancer increasingly benefits from research and technological advances, for example mutated c-kit targeted therapy in leukaemia and gastrointestinal tumours, although not necessarily curative certainly offer hope for prolonged survival.[1] For other malignancies these advances have not yet heralded comparable benefits, a case in point being uveal melanoma (UM). UM is the most common primary intraocular malignancy of adults, with tumours arising in the Iris, ciliary body and choroid. Approximately 5 - 7 cases per million population are diagnosed annually.[2] Iris melanomas are relatively benign, but posterior UM (ciliary body and choroid) still present enormous challenges, and despite successful and conservative treatment of primary tumours, survival rates over the last 25 years remain unchanged.[2-4] Metastasis invariably targets the liver, and the detection of hepatic lesions signifies a dismal outcome, with median survival only 6 months.[5] There has been a slight improvement recently in survival rates following detection of hepatic metastases, possibly reflecting earlier detection,[5,6] due in part to research that has established how to reliably determine those patients that will die usually within 5 -7 years. This categorization depends on the detection of genetic changes of chromosomes 3 and 8.[7-10] Thus a very thorny problem arises, that although we can reliably identify patients with the poorest outcome, there is very little to be offered for their effective further treatment. For a woefully small percentage of patients surgical resection of hepatic metastases and liver embolization have achieved remarkable successes.[5,11] What hope though for the future treatment of most patients with UM?

Uveal and cutaneous melanomas are genetically dissimilar.

Cancer therapy relies upon the toxic effects of agents targeting replicating cells, thus being proportionally more detrimental to the rapidly dividing cancer cell population. Recently effective cancer treatments have targeted features of cancer cells not usually associated with normal cells; For example activation of the Mitogen - Activated Protein Kinase / Extracellular-signal Related Kinase pathway (MAPK/ERK pathway known by other names as well). The MAPK pathway is essential in mediating cell cycle progression, and mutations in this pathway result in it being constitutively activated in several types of cancer, producing inappropriate proliferation.[12,13] Mutations of *BRAF* and *RAS* activate the MAPK pathway, through stimulation of mitogen-activated protein kinase kinase or MEK (also known as *MAP2K* and *MKK*), and are present in 66% and 15% of cutaneous melanomas (CM) respectively.[14,15] As mutations of *BRAF* and *RAS* are almost mutually exclusive it suggests both independently regulate the MAPK pathway.[16] Deregulation of the MAPK pathway is also seen in UM; however *BRAF* mutations are rarely seen amongst non-cutaneous melanomas such as UM,[17-20] and only then affecting a minority cell population.[21] As downstream targets of the MAPK pathway are constitutively activated in UM, a different mechanism implicating other genes appears to disrupt the MAPK pathway.

Despite arising from the same cell type, there are more dissimilarities in the genetics of UM and CM than similarities.[22] Approximately half of UM have monosomy 3 and additional copies of the long arm of chromosome 8 ,[7,22,23] and the presence of these changes consistently amongst aggressive UM, is reliably used to predict prognosis.[7-10,22-24] Similar predictive alterations are not found in CM. There is

some common ground between the two forms of melanoma, as both share alterations affecting comparable regions of chromosomes 1 and 6, however other recurrent alterations have been described in CM that are quite different.[22,25] Furthermore genes implicated in CM are not affiliated to both forms. For example, germ-line mutations in *p16/INK4a* result in an autosomal dominant predisposition to CM,[26,27] but not necessarily to UM,[28,29] where inactivation of *p16* through methylation appears to have a role.[30] These findings suggest that although genetic alterations are shared by both forms of melanoma, the frequency, and therefore the reliance placed on them to advance CM and UM development is dissimilar.[22] If the MAPK pathway is activated in both, but *BRAF* and *RAS* are not the targets in UM, what is?

The heterotrimeric G protein α subunit (GNAQ) and uveal melanoma

Little is known about the molecular pathogenesis of UM; but recent work has highlighted a role for the heterotrimeric Guanine nucleotide-binding protein G(q) subunit alpha (GNAQ, or G-alpha-q).[31,32] GNAQ is one of a subfamily of genes, comprising *GNAQ*, *GNA11*, *GNA14* and *GNA15/16*. [33] Activating mutations of *GNAQ* occur in approximately half of UM, almost all blue naevi, 27% of cases of Nevus of Ota and some melanomas of the nervous system.[31,32,34] The *GNAQ* mutation is somatically acquired arising exclusively in exon 5 at codon 209 resulting in substitution of the original glutamine at this point. There are at least 5 known variants, most frequently resulting in either GNAQ^{Q209L} or GNAQ^{Q209P}. [32] Mutations of codon 209 have also been recently found in *GNA11*, and both *GNAQ* and *GNA11* can also have mutations of exon 4 affecting codon 183.[35] Thus, over 80% of UM in a recent study were found to have either *GNAQ* or *GNA11* mutations.[35]

In contrast *GNAQ* mutations in CM are rare, as indeed they are amongst other non-melanocytic tumours.[32,36,37] There is not however a clear cut division since conjunctival melanomas often have *BRAF* involvement, but do not have *GNAQ* mutations,[38] and amongst UM the relatively benign anterior Iris melanomas less often have *GNAQ* mutations, but can occasionally have mutant *BRAF*.[31,32,39] Taken together, these observations suggest that activation, and possibly regulation, of the MAPK pathway in the melanocyte lineage may be split into two branches, with more reliance placed on *BRAF* by CM/melanocytes, but with *GNAQ* more relevant to UM and some other non-cutaneous melanoma/ melanocytes.[32] There is however no direct evidence to suggest that *BRAF* and *GNAQ* mutations are functionally equivalent, and *GNAQ* mutations could have a different role in UM development. Mutations of *GNAQ* are not correlated with tumour stage, chromosomal aberrations or other clinical features indicative of poor outcome,[40] but as they are present in all stages of progression it is suggested that they may be initiating or early events.[31] A premise that fits well with a purported role in activating the MAPK pathway, representing early deregulation of proliferation as key to some UM. If this is the case, how would *GNAQ* mutations serve to regulate UM proliferation?

Role of *GNAQ* in activated G-Protein Coupled receptor (GPCR) signalling

Uncontrolled proliferation is a key feature of malignant transformation and activation of the MAPK pathway is a common target for malignant progression. Any functional deregulation, through increased stimulation by growth factors, or mutations and amplifications of the genes in the pathway, can effectively induce tumour growth; hence hijacking of these regulatory pathways is an essential mechanism whereby

cancers can auto-regulate their own growth.[41] Signals from growth regulators are transmitted by a large family of transmembrane receptors, known as G-Protein Coupled Receptors (GPCR).[42] Effective intracellular signalling from GPCR requires activation of Phospholipase C via a heterotrimeric or 'large' G-protein complex.[42] It is here that GNAQ is active as an alpha sub-unit of the heterotrimeric complex that activates Phospholipase C. This complex also consists of G β and G γ sub-units, and its sole task is to couple extracellular ligand receptor binding signals to the intracellular signal-processing network. In the absence of signalling, the heterotrimeric G α - β - γ complex remains bound to the receptor and G α is present in a GDP-bound state. In the presence of ligand binding, G β and G γ activate Src, and phospholipase C β , leading to Raf and Ras signalling.[43]

How could *GNAQ* mutation promote tumour progression?

The potential proto-oncogenic nature of GTPase deficient mutants of several G alpha sub-units has been recognised for a number years.[44-46] Under normal circumstances dissociation of GNAQ in its GTP-bound state specifically activates Phospholipase C β . [43] Mutations of *GNAQ*^{Q209L} result in loss of GTPase activity producing constitutive activation of *GNAQ*. [47] This has the same effect as dissociation of *GNAQ* in its GTP-bound state leading, via Ras and Raf signalling, to MEK/ERK (MAPKK/MAP2K/MKK and MAPK1 respectively) activation.[41] Activation of down-stream effectors of GPCR can thereby bypass growth factor dependency, and in CM, mutations of *RAS* and notably *BRAF* result in ERK1/2 mediated Fos and Jun activation and cell cycle progression.[17-19] For UM growth factor independence could be achieved by failure of mutant *GNAQ* to form the heterotrimeric large G-

protein complex, producing uncoupling of the receptor and constitutive activation of G-protein signalling.[31,32] Thus *GNAQ* could constitutively activate growth pathways, and indeed in mice with mutant *GNAQ* or *GNA11*, where they are compensatory, an accumulation of non-epidermal (dermal) melanocytes arises.[48] As it has recently been found that mutually exclusive mutations of *GNAQ* and *GNA11* occur in over 80% of UM it seems likely that a similar compensatory role also exists for UM.[35] No direct association between *GNAQ* mutations and increased proliferation in UM has been found, but mutations of *GNAQ* do produce spontaneously metastasizing melanomas in mice.[35,49] Possibly for *GNAQ* mutations to be effective, interaction with other impaired or deregulated genes is required. For example mutated *RAS* alone is unable to induce malignant transformation, but activation of Ras signalling is sufficient to transform normal cells, therefore the synergistic activation of other pathways, notably p53 is required.[50] A comparable situation may also exist for *BRAF* mutations in CM, whereby activating mutations do not always directly correlate with increased proliferation.[51] The signalling pathways that synergise with *GNAQ* (or *GNA11*) mutations are unknown and there is no evidence for other members of related signalling pathways being mutated in UM.[31] There is much to learn regarding the action of *GNAQ*, but given the high frequency of *GNAQ* (and *GNA11*) mutations in UM and amongst other non-cutaneous melanomas it suggests that in some manner *GNAQ* undertakes a fundamental role in tumour development.

Origin and differentiation of melanocyte populations: Relevance to melanocytic neoplasms.

Early studies implied that *GNAQ* may be differentially implicated amongst melanocytic populations, as *GNAQ* mutations produced hyperpigmented skin in mice by increasing intradermal melanocytes; but did not affect epidermal melanocytes.[48] With the exception of Retinal Pigment Epithelial cells, all melanin-producing cells are derived from the neural crest and during development melanocyte progenitors migrate to various locations.[48] It is thought that in mice there are location-specific distinctions centred on melanocytes being cutaneous as opposed to non-cutaneous.[52] Response to specific differentiation signals was dependent on location, with melanocytes of non-cutaneous sites being less sensitive, via signalling through the tyrosine kinase receptor Kit, to stem cell factor (SCF) binding.[52] Instead, non-cutaneous melanocytes, were preferentially stimulated by Hepatocyte Growth Factor (HGF) and Endothelin 3 (ETB-3).[52] It is plausible that tumours derived from these distinct populations likewise are dissimilar in their response, despite prevailing on the same growth regulatory MAPK/ERK pathway. The initiating events that drive cutaneous and non-cutaneous melanocytes to form tumours are therefore likely to be diverse, hence partitioning of *BRAF* and *GNAQ* mutations amongst melanomas.[14,15,31,32] It is perhaps pertinent that non-cutaneous melanomas respond preferentially to HGF, as this growth factor is one of the most stimulatory of UM.[53,54] HGF activates the MAPK/ERK pathway,[55] so *GNAQ* mutations could circumvent a requirement for HGF, or perhaps synergize with HGF to constitutively activate the MAPK/ERK pathway. Given what is known about the role of *GNAQ* in UM, is there any potential to capitalize on its association?

GNAQ mutations as a therapeutic target?

Recent trials in CM, using B-Raf inhibitors, have shown some remarkable successes.[56,57] As *GNAQ*^{Q209} mutations are exclusive to melanocytic tumour cells, they represent a similarly promising target for UM, but there are issues. . Firstly, whether the number of patients that could benefit is sufficiently large enough to warrant the large commercial investment required. Secondly *GNAQ* activity is crucial for cardiomyocyte survival, over-expression of *GNAQ* induces cardiomyocyte hypertrophy, apoptosis and cardiac failure, whilst in contrast knock-out of *GNAQ* induces cardiac hypoplasia in embryonic mice.[58,59] Alternatively inhibitors could be designed that block mutant *GNAQ* protein, whilst leaving wild-type *GNAQ* protein unaffected. If targeting *GNAQ* proves problematic inhibitors that counteract aspects of the MAPK/ERK pathway, such as those of B-Raf, Ras, MEK and ERK may be equally effective, and initial data suggests MEK inhibitors have potential value for UM. [35] Finally other mutations, such as those recently identified of *BAP1* in UM may ultimately prove of more value.[60] Mutations specific to UM, such as those of *GNAQ*^{Q209} and *BAP1*, although not offering the panacea, do at least point a way forward.

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