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Editorial

The Cancer Genome Atlas Project: An Integrated Molecular View of Uveal Melanoma

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Uveal melanoma (UM) is the most common primary intraocular tumor in adults. It can be treated by radioactive plaque, proton beam or stereotactic irradiation, or enucleation. Despite excellent local control, up to 50% of patients will die of metastases.[1] Unfortunately, there is currently no effective treatment for metastatic UM. A wide range of tumor characteristics is associated with the risk of these metastases developing, and several tests using tumor DNA or RNA can be applied to determine whether a tumor is likely to metastasize. Knowledge about the parameters involved in the development of metastases helps us to understand the basic mechanisms behind the metastatic process. This information one day may contribute to finding an effective treatment that either will prevent metastases or cure them if they occur. Although in recent years new treatments, such as targeted chemotherapy or immunotherapy, have proven successful in many types of cancer, these have not worked in UM. Apparently, UM has some distinct and unusual features. The Cancer Genome Atlas (TCGA) project was set up to study the characteristics of many different types of cancer, and recently focused on UM. This has led to better insight into the genetic and immunologic make-up of this malignancy, helping to categorize it into 4 main groups that differ prognostically.[2]

Knowledge about the parameters involved in the development of metastases helps us to understand the basic mechanisms behind the metastatic process.

Cancer is a disease of mutations and chromosome aberrations that lead to uninhibited cell division. Usually, multiple mutations are needed for cancer to develop. In the development of UM, mutations in the *GNAQ* or *GNA11* genes are considered an early event.[3,4] Mutations in these genes activate several molecular pathways, setting the stage for progression toward cancer. We recently showed these mutations already are present in nevi, confirming their role as very early events.[5] However, only 1 in 9000 nevi will progress to melanoma, and different hypotheses exist regarding the sequence of events that follows the *GNAQ* or *GNA11* mutations, thus transforming a lesion into malignancy.[6] Subsequent changes may involve chromosome changes, mutations, or both. It is already known that some of these are related to prognosis in UM. Loss of 1 chromosome 3 and having additional copies of chromosome 8q are associated with worse survival (reviewed by Dogrusöz and Jager[7]), whereas additional copies of chromosome 6p predict a better prognosis. Several mutations are associated strongly with the development of metastases, as is the case with mutations in the *BAP1* gene,[8] or only with the late development of metastases, as was recently reported for mutations in the *SF3B1* gene.[9,10] Although chromosome status as well as mutations are being used for prognostication, messenger RNA (mRNA) expression may be used as well.[11] Different so-called mRNA gene expression profiles are associated with a low versus a high risk of metastasis formation, and this has led to the availability of commercial tests for prognostication.[12,13] This prognostication can be used to include high-risk patients in screening programs for early detection of metastases or in trials for adjuvant treatments.

The Cancer Genome Atlas project was an enormous undertaking; it was set up and supported by the National Cancer Institute in the United States with the goal of determining the molecular identity of many types of cancer and generating ideas about the comparative biologic features of malignancies. Recently, TCGA project focused on UM.[2] Primary tumor material from 80 patients with UM was collected. Various tests were performed, analyzing histologic features, chromosome copy numbers, genetic mutations, expression of RNA, proteins, DNA methylation status, and other factors such as biochemical pathways and immune markers. Results from these different tests (also known as *platforms*) were compared and related to clinical outcome. This TCGA project study led to an overall view regarding UM and helped us tremendously in integrating the different pieces of the puzzle. In short, the outcome is that almost all patients with UM carry a mutation in the *GNAQ* or *GNA11* gene and that application of the different platforms leads to the identification of 4 main types of UM. In the following sections, the major findings of TCGA project on UM are discussed, providing an integrated molecular view of this disease.

Prognostic Groups

The overall conclusion of the various analyses in TCGA project was that UM are quite consistent in their molecular patterns: with each platform, only 2 to 5 groups were

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identifiable. Overall, 2 main groups were identified; these were defined by the presence or absence of 2 chromosomes 3 (commonly referred to as disomy 3 [D3] or monosomy 3 [M3], respectively). Both of these groups again could be divided into 2 subsets with their own characteristic genomic, signaling, and immune profiles. In the TCGA project marker article on UM, the 4 main prognostic groups were named 1, 2, 3, and 4, but to prevent confusion with Castle Bioscience's Class 1/Class 2 (a commercially available test based on gene expression), and following the nomenclature introduced in an article on genetics in UM,[14] we refer to the 4 groups as A, B, C, and D.

Copy Number Analysis

Most studies that looked at the chromosome constitution of UM have shown that loss of 1 chromosome 3 and addition of copies of 8q in the UM cells are associated with an increased risk of metastases.[7] When chromosome copy numbers were determined (by single nucleotide polymorphism SNP microarray and whole exon sequencing), unsupervised computer analysis of somatic copy number alterations identified clusters of samples with shared features. This led to 4 subtypes of UM, dividing D3 as well as M3 tumors into 2 subgroups, that is, A and B versus C and D. Although A and B groups both carry 2 chromosomes 3 (D3), they differ in their chromosome 6 and 8 status: group A shows enrichment for partial or total 6p gain without other chromosome aberrations, whereas group B shows both 6p gain and partial 8q arm gain. As already mentioned, groups C and D are M3 tumors, with most showing 8q whole arm gain. Most of the cluster D tumors had an isochromosome for 8q, leading to multiple copies of 8q.

Mutation Analysis

Whole exon sequencing, a relatively new technique to analyze a wide range of genes in 1 test, revealed there are very few somatic mutations in UM. Similar to prior studies, mutually exclusive somatic mutations were present in the G protein pathway-associated *GNAQ* or *GNA11* genes (92.5%), with further mutations observed in the *CYSLTR2* gene (4%) and *PLCB4* gene (2.5%). As mentioned before, the *GNAQ* and *GNA11* mutations already occur in most choroidal nevi, so they do not define malignancy.

In addition to these primary mutations, almost all tumors contained a second mutation. The most important secondary mutation occurred in the *BAP1* gene and was associated with the separation into prognostically favorable (A and B) or unfavorable (C and D) tumors. The *BAP1* gene, located on chromosome 3, encodes the BAP1 protein (i.e., BRCA1-associated protein 1) and has a role as tumor suppressor. BAP1 expression was normal in most D3 (A and B) tumors and downregulated in most M3 (C and D) tumors.

Tumors with *BAP1* gene mutations showed a low level of *BAP1* mRNA, but because no immunohistochemical stainings were planned for this project, we do not know whether these tumors expressed BAP1 protein, which previously was shown to be a reliable prognostic marker.[15] Analysis of genetic clonality suggested that loss of 1 chromosome 3 preceded loss of *BAP1* through mutation. *BAP1* gene alterations were shown in 83% of the M3 UM cases. As the other 17% showed a decreased expression of *BAP1* mRNA, further tests may detect a mutation or epigenetic downregulation in the *BAP1* gene in those tumors. The M3 *BAP1* loss tumors showed a different overall methylation pattern compared with the D3 *BAP1*-expressing tumors, suggesting that loss of *BAP1* (or potentially another gene on chromosome 3) causes a wide range of epigenetic modifications.

Other secondary mutations were identified in the *SF3B1* and *EIF1AX* genes, and these mutations were nearly mutually exclusive with *BAP1* mutations. Mutations in *SF3B1* or *EIF1AX* occurred in 28 cases (34%) of TCGA project panel. All 10 *EIF1AX* mutations occurred in group A, and most of the *SF3B1* mutations (13/18) occurred in group B. *SF3B1* mutations in D3 UM were associated with partial 8q gain.

A novel mutation in *SRSF2* was detected in 3 UM samples studied in TCGA project. Tumors with *SRSF2* mutations had neither *SF3B1* nor *EIF1AX* mutations and were found in both D3 and M3 UMs with 8q gains. These findings suggest functional similarities between *SRSF2*- and *SF3B1*-mutant UM. The D3 *EIF1AX*- and *SF3B1*- or *SRSF2*-mutated tumors had their own distinct methylation patterns.

Analysis of mRNA, Micro RNA, and Long Noncoding RNA

Various types of RNA were analyzed in TCGA project: mRNA, micro RNA (miRNA), and long noncoding RNA (lncRNA). Although mRNA is the template of DNA used to produce proteins, miRNA and lncRNA do not result in proteins, but rather have a role in regulation of various transcription processes. Expression analysis of the various RNA types resulted in 4 distinct groups of UM, which is in contrast to the previously published 2 or 3 prognostic groups based on gene expression profiling. Using gene expression, Tschentscher et al[12] were the first investigators to show 2 distinct classes of UM. Onken et al[13] similarly identified 2 classes, known as class 1 and class 2, which are used in the Castle Bioscience test. The Cancer Genome Atlas project showed that the chromosome 3 status determines the grouping of mRNA, as expected: 6 of 8 genes that are high in Onken's class 1 and low in class 2 are located on this chromosome. The 2 gene expression classes follow in almost all cases the distribution of chromosome 3, with class 1 corresponding to D3 (tumor types A and B) and class 2 corresponding to M3 (tumor types C and D). In the TCGA project study, *BAP1* mRNA expression followed the same pattern with loss of expression occurring in M3 tumors. Based on the mRNA expression pattern, both the D3 as well as the M3 tumor group were divided further into 2 groups. This division did not follow the distribution of the *SF3B1* or *EIF1AX* mutations, as also previously seen with the 4 groups according to their somatic chromosome copy numbers. Analysis of lncRNA expression patterns similarly identified 4 subgroups, which paralleled the same 4 subgroups identified by mRNA expression pattern, and the chromosome copy numbers.

Table 1. Overview of Types of Uveal Melanoma and Corresponding Chromosome Aberrations

	A	B	C	D
mRNA class	1	1	2	2
Chromosome aberrations	Infrequent	Infrequent	Frequent	Frequent
Chromosome 3	Disomy 3	Disomy 3	Monosomy 3	Monosomy 3
Chromosome 6	Extra 6p	Extra 6p		
Chromosome 8	Normal 8q	Partial extra 8q	Extra 8q	Extra 8q (multiple)
Inflammation	None	None	Some	Much
Prognosis	Favorable	Late metastases	Unfavorable	Unfavorable

One group in TCGA project (i.e., group D), which was identified with mRNA and lncRNA heatmaps (a clustering technique based on gene expression levels), displayed a specific peculiarity: this group contained most of the tumors with an increased leukocyte fraction. Although a T-cell infiltrate was nearly absent in D3 UM (groups A and B), it was present in approximately 30% of M3 UM (groups C and D), with a remarkable abundance in group D. Genes involved in interferon signaling, cytotoxicity, and immunosuppression also were expressed specifically in this group D. The Cancer Genome Atlas project study confirmed the finding that M3 tumors have a higher HLA expression than D3 tumors.[16] This would make these tumors theoretically more susceptible to immune therapy, because a proper HLA antigen expression is essential for tumor cell recognition by T cells. However, it may be that either the immune-privileged environment of the eye or the presence of regulatory elements in the infiltrate (with FOXp3+ expressing regulator T cells, or expression of the indoleamine 2,3-dioxygenase (IDO) enzyme that suppresses T-cell responses) could inhibit the induction of systemic effector cells.[17–19] Another reason for the lack of successful treatment with immune checkpoint inhibitors in UM may be the relative lack of neoantigens, which play a role in developing T-cell responses.[20] Neoantigens are antigens on tumor cells formed by newly mutated genes. Because they are absent normally, they are seen as foreign and form a potential target for T-cell attack. The relative lack of mutations associated with UM compared with other cancers (for example, cutaneous melanoma) may explain why there are so few neoantigens in this tumor.[21]

Pathway Analysis and the Role of 8q

Pathway analysis showed differentially expressed pathways in groups C and D. One of the 2 M3 clusters expressed DNA damage–response genes *HIF1a* and *MYC*, as well as expressed immune-related genes. The other M3 cluster showed an increased expression of *MAPK* and *AKT*.

When looking for mRNA markers in groups C and D that differentiate between patients with and without metastases, a number of genes were identified. Most of these were located on chromosome 8q. The association between the presence of extra copies of chromosome 8q and the development of metastases has been shown in several studies and has been observed to be independent of the chromosome 3 status. The specific genes involved have not yet been identified, however. Recently, an association between extra copies of chromosome 8q and infiltration with monocytes, but not T cells, was observed in D3 *BAP1*-positive tumors.[22] The same study showed an association between an increase of infiltrating T cells with M3 and loss of expression of *BAP1*. We assume this influx of T cells, upregulation of the interferon-signaling pathway, and increased HLA antigen expression are related to the deubiquitinase activity of *BAP1*, because such an immune regulatory function has been described for other deubiquitinases as well.[23]

Sequence of Mutational Steps

Combining the aforementioned data, 4 groups of UM can be identified that are characterized by different RNA expression profiles, chromosome aberrations, and prognosis (Table 1). It can be deduced that the first step in the development of UM is a mutation in the *GNAQ* or *GNA11* genes, whereas the subsequent step may either be a mutation in the *SF3B1*, *EIF1AX*, or similar gene, or addition of an extra copy of chromosome 8q. It is as yet unknown what happens next. Although TCGA project study suggests that loss of 1 chromosome 3 precedes the development of a *BAP1* mutation, it would be more logical when considering cancer in general that the *BAP1* mutation precedes the chromosome loss. We and others recently reported that the addition of chromosome 8q precedes the loss of 1 chromosome 3.[24,25] It is as yet unknown which genes on chromosome 8q play a role in cancer progression.

Different groups have demonstrated that specific chromosome changes, such as loss of 1 chromosome 3, are associated with specific gene expression patterns.[12,26] The multiplatform approach used in TCGA project clearly shows that the mutations in *EIF1AX*, *SF3B1*, and *BAP1* are related to specific chromosome aberrations and that these lead to specific patterns in mRNA, miRNA, lncRNA, and epigenetic changes such as methylation patterns. Using these different platforms, 4 groups of UM were identified instead of the only 2 or 3 prognostic groups reported previously and tested for commercially. Furthermore, TCGA project study convincingly attributed a very important role to *BAP1*, both in the development of an inflammatory phenotype, as well as in the development of metastases. The molecular pathways remain to be elucidated.

Clinically, it is clear that one can use either the type of mutation; chromosome aberrations; or mRNA, miRNA, or lncRNA analysis to determine prognosis. Scientifically, more work remains to understand why the different chromosome changes play such an important role in the biological

progression of UM and how we can modify the pathways to prevent or treat metastases. Finding the targets on chromosome 8q that play a role in metastases and tumor progression may be easier than overcoming loss of function changes through loss of chromosome 3 and expression of *BAP1*.

In summary, TCGA project was a massive undertaking and has added a great deal to our understanding of the molecular underpinnings of many cancers including UM. The large amount of data generated in the UM TCGA project and published in the TCGA marker article[2] has paved the way for many investigations and further analyses that have followed, and likely will continue to serve as an invaluable resource for UM investigators in the years to come.[27]

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Footnotes and Financial Disclosures

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