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Review

Molecular and metabolic features of oncocytomas: Seeking the blueprints of indolent cancers☆

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ABSTRACT

Oncocytic tumors are a peculiar subset of human neoplasms in which mitochondria have been proven to have a prominent role. A number of paradoxes render these clinical entities interesting from the translational research point of view. Most oncocytic tumors are generally metabolically constrained due to the impaired respiratory capacity and lack of the ability to respond to hypoxia, yet they maintain features that allow them to strive and persist in an indolent form. Their unique molecular and metabolic characteristics are an object of investigation that may reveal novel ways for therapeutic strategies based on metabolic targeting. With this aim in mind, we here examine the current knowledge on oncocytomas and delve into the molecular causes and consequences that revolve around the oncocytic phenotype, to understand whether we can learn to design therapies from the dissection of benign neoplasms. This article is part of a Special Issue entitled Mitochondria in Cancer, edited by Giuseppe Gasparre, Rodrigue Rossignol and Pierre Sonveaux.

1. Introduction

Oncocytic tumors are a subset of epithelia-derived peculiar neoplasms in the pathogenesis of which mitochondria stand out as being inevitably involved. Generally arising in endocrine and exocrine organs, oncocytic tumors display one of the most bizarre cytologies, as they are composed of swollen cells (i.e. the oncocytes) packed with mitochondria, while the nucleus is small and the rest of intracellular organelles is difficult to distinguish within the eosinophylic cytoplasm [1]. Often, oncocytes' mitochondria appear deranged with aberrant or even absent cristae, showing low electron density on micrographs, indicating that they are most likely not functional. Such peculiar morphologic features have prompted investigators to explore the role of mitochondria and their related metabolism in oncocytic tumors, as pathologists were often called to face a clinical entity that clustered apart from other neoplasms in terms of molecular profile, therapy response and outcome [1–3].

Whether oncocytic tumors arise in the thyroid, in the kidney, in the parathyroid, in the pituitary or in the salivary glands, their cytology is very similar and their histology recognizable via hematoxylin/eosin staining, corroborated by electron microscopy or a specific staining against mitochondrial proteins [1]. Generally, oncocytic tumors share the feature of being less aggressive than their non-oncocytic counterparts [2]. The exceptions occur in a few cases, particularly in the thyroid, where tumor heterogeneity remains a cogent issue in predicting the outcome, and where prognosis is given independently from the occurrence of oncocytic phenotype [4]. Furthermore, oncocytic tumors harbor high burden of disruptive mutations in mitochondrial DNA (mtDNA)-encoded genes for respiratory chain subunits, which likely accounts for their deranged mitochondrial morphology/structure. Such genetic markers are thus far the only lesions unequivocally associated to the oncocytic phenotype, regardless of the organ where they occur. Conversely, mtDNA mutations are only seldom withstood by non-oncocytic cancer cells, and therefore quickly selected against [5,6]. Since oncocytic tumors carry mutations in metabolic enzymes, i.e. the respiratory chain complexes, their in-depth molecular and biochemical investigation is warranted, as they potentially hold a bulk of information on the metabolic rewiring tumor cells undergo to survive, which is a hallmark of cancer [7].

Several paradoxes make these neoplasms interesting from the translational research point of view. Owing to their respiratory impairment,

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oncocytic tumors are, generally, metabolically constrained Moreover, they lack the ability to respond to hypoxia, and to easily acquire a highly glycolytic capacity driven by hypoxia-inducible factor 1a (HIF1-α) [8,9]. Nonetheless, they maintain features that allow them to strive and persist in an indolent form, which somewhat resembles senescence or dormancy. However, despite a profound bioenergetics crisis, they do not represent a complete failure of tumorigenesis. Interestingly, oncocytic tumors appear to favor selection of mutations that are instead purified in aggressive cancers [5,6], a process that may be only partly explained by random drift, as mutations in the respiratory chain have a marked functional significance. The mechanisms underlying this homoplasmic shift of pathogenic mtDNA mutations in oncocytomas is still unknown. The typical mitochondrial hyperplasia of oncocytic tumors suggests that the balance between mitochondrial biogenesis and auto/mitophagy may play a pivotal role [10,11]. Furthermore, the contribution of specific patterns of oncogenes activation and tumor suppressor genes (TSGs) inactivation, along with the action of modifier genes, might explain the accumulation of mtDNA mutations in oncocytomas. It is plausible that oncocytes may find advantages in their metabolic crisis during certain stages of tumor progression, and recent evidences highlight the involvement of dormancy associated to therapy resistance [3,12,13]. Overall, it appears clear that a balance of several factors must be reached in order to achieve the oncocytic phenotype: i) a lesion hitting the mitochondrial energy production capacity, which triggers bioenergetics crisis; ii) an imbalance between mitochondrial biogenesis and autophagy/mitophagy, which may predispose to the accumulation of damaged organelles, and iii) the contribution of oncogenes/TSGs/modifier genes to the onset of the previous two conditions.

Within the dynamic picture of cancer progression, it is yet unclear whether a tumor arises as oncocytic or acquires such phenotype due to secondary hits following the primary transformation. The second hypothesis appears more plausible as oncocytes are mostly low-proliferating. However, the occurrence of fast-growing oncocytic tumors has been reported and light must be shed on this matter. Particularly in few hereditary cases, a subgroup of oncocytic neoplasms does not show mitochondrial aberrations despite hyperplasia [14,15]. Whether absence of mtDNA mutations may explain the divergent clinical behavior, associated to the diverse metabolic features of these tumors, is still a matter of investigation. Identification of the mechanisms causing oncocytic tumors, along with the thorough dissection of the pathways deregulated in benign oncocytomas, may reveal novel ways for therapeutic strategies.

In this review, we examine the current knowledge on these unusual and underdiagnosed human neoplasms and delve into the molecular causes and consequences that revolve around the oncocytic phenotype. We attempt to shed light on the numerous mysteries surrounding the bioenergetics of mtDNA-mutated cancers, and draw potential patterns of nuclear-mitochondrial crosstalk, with the intention to understand whether it is feasible to design anti-cancer therapies by dissecting benign neoplasms.

2. CI mitochondrial DNA mutations as a hallmark of oncocytic transformation

Somatic non synonymous mtDNA mutations usually associate with older age [16]. In cancer, mtDNA mutations occur as relatively rare events. Severe mtDNA mutations are purified from human cancers and usually found at low heteroplasmy levels [6,17,18]. Unexpectedly, oncocytomas often harbor highly severe mtDNA mutations [5], which may completely inhibit oxidative phosphorylation (OXPHOS). For instance, low heteroplasmy levels of a *MT-ND5*-truncating mitochondrial mutation were described in non-oncocytic cancers, such as hepatocellular carcinoma, renal, breast, gastric, rectal and ovarian cancer [5,19,20], yet the homoplasmic variant of the same mutation is only found in oncocytic tumors, such as in a peculiar case of nasopharyngeal oncocytoma [21]. As a consequence of the accumulation of deleterious mtDNA mutations, which cause a bioenergetics crisis in the cells, oncocytic tumors may not be able to progress to malignancy and remain mostly confined as low proliferative lesions [5,17].

MtDNA mutations may be induced by several mechanisms. They may be a consequence of a defect in nuclear genes involved in mtDNA replication, like *POLG* [22], which are considered responsible for many mitochondrial disorders [23]. Reactive oxygen species (ROS) generated by mitochondrial oxidative chain may lead to mtDNA damage (Fig. 1(3)) [18], as well as an altered nucleotide biosynthesis or impaired nucleotide transport between the cytosol and the mitochondrial matrix, as in the case of adenine [23]. Worth mentioning are the exogenous sources of mtDNA mutations such as ultraviolet light, ozone, ionizing radiations, metals, pesticides, air pollutants or pharmaceutical drugs, asbestos, and arsenic [24,25]. Some chemotherapy treatments may have off-target effects on mitochondria, via induction of mtDNA damage. Notably, cisplatin, bleomycine and neocarzinostatin are all ROS-producing mutagens [26,27]. The causative agents for mtDNA mutations in oncocytic tumors are yet to be uncovered. It may be hypothesized that any initial mtDNA mutation, even at low loads, may contribute to increase ROS levels, further triggering the occurrence of additional mutations in a feed-forward loop (Fig. 2(1)) [28].

The involvement of mtDNA defects in oncocytic tumors was reported for the first time in 1989, when two independent groups showed abnormal mtDNA restriction fragment pattern exclusively in renal oncocytoma [29,30], which usually appears histologically homogenous and with a favorable prognosis/outcome [31]. Subsequently, whole mtDNA sequencing allowed the identification of mtDNA mutations in the vast majority of renal oncocytoma cases [32–34]. The mutations were mostly found in genes encoding subunits of respiratory chain complex I (CI) and, in at least 60% of cases, these were stop codon or frameshift mutations known to disrupt the complex assembly [33,34]. Occurrence of mtDNA mutations affecting directly or indirectly CI explained the marked decrease in enzymatic activity, which was the main biochemical hallmark of renal oncocytoma [35]. Somatic mutations in the mtDNA of thyroid oncocytic tumors are found in 50–60% of patients. Unlike the mutations found in renal oncocytomas, which are mainly disruptive, mtDNA changes in oncocytic tumors of the thyroid are more often missense. In more detail, from 75 mutational events reported in thyroid oncocytic tumors by different groups [32,34,36], 50% induced an amino acid change, whereas 26% were frameshift or nonsense mutations affecting CI assembly. Interestingly, the m.3571insC mutation in *MT-ND1* has been detected in several cases, as well as in the only existing oncocytic cell line model XTC.UC1 [37], where it has been shown to disrupt CI and exhibit anti-tumorigenic effect only when present above 83% mutant load threshold [38]. The studies analyzing mitochondrial genome in parathyroid and salivary gland oncocytomas also reported high frequency of mtDNA mutations. In parathyroid, they were found in approximately 60% of cases [34,39], whereas in the salivary glands in 80% of cases [5,8,34]. Oncocytic cells are also found in normal tissue, such as in parathyroid where they may coexist together with clear chief cells. A comparative study comparing these two subtypes both in pre-oncocytic and oncocytic parathyroid lesions, showed that the oncocytic phenotype significantly associates with COX deficiency or CI point mutations, respectively. Such mitochondrial impairment was accompanied by an increased mtDNA copy number indicating enhanced mitochondrial biogenesis [40]. All together, these findings underline that respiratory defects are distinctive features of oncocytic phenotype, regardless of cell transformation or the tissue of origin.

Whether a mtDNA mutation will accumulate and be stabilized in a tissue mainly depends on putative selective pressures [41]. In this regard, we may hypothesize that a positive selection during hyperplasia/tumor progression could explain the homoplasmic nature of mtDNA mutations in oncocytomas. However, this appears paradoxical since these mutations induce a severe bioenergetic crisis. Noteworthy, a mathematical model showed that homoplasmic shift might also be a

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