**Letter to the Editor**

**Adipophilin expression in primary and metastatic uveal melanoma: a pilot study**

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Dear Editor,

Adjustment of cancer cell metabolism to enable rapid cell growth and division is firmly established as one on the hallmarks of cancer. An alteration associated with this metabolic phenotype is an upregulation of lipogenic pathways, characterized by high lipid droplet (LD) content in the cytoplasm of the cancer cells. LDs consist of a triacyglycerol core with phospholipid monolayer on the surface, in which amphiphilic proteins insert. The perilipin, adipophilin (ADP), tall-interacting protein of 47 kDa (PAT) family is crucial for the formation, modification and involution of LD [1].

Interference with PAT proteins, such as ADP, may have an important metabolic or antineoplastic effect on tumor cell viability. The analysis for PAT proteins has been conducted only in a few tumor entities, such as lung carcinoma, breast carcinoma, Burkitt lymphoma and very recently in cutaneous melanoma [2, 3]. The value of ADP has already been validated as a diagnostic biomarker in sebaceous carcinoma [4]. Our incidental observations of lipid droplets in UM cell lines (Figure) and in some primary uveal melanomas (UM) led us to undertake this pilot study in which we evaluated the expression of ADP in UM, and comparing this with the histomorphological and genetic features of the tumors.

Immunohistochemical analysis of ADP expression was performed in 34 consecutive enucleation specimens from 22 male and 12 UM female patients. These comprised 22 choroidal (64.7%) and 12 (35.3%) ciliochoroidal UM. The intensity of staining (IS) and the proportion of tumour cells stained (PS) for ADP were evaluated semiquantitatively. The IS was graded as weak (1), moderate (2), strong (3) and intense (4). The PS of ADP was scored as 0-24% (1), 25-49% (2), 50-74% (3) and >75% (4). The total expression of ADP (tADP) was obtained by multiplying IS and PS scores together. Results were compared with clinical and histological parameters (Table 1). In addition, 5 UM liver metastases were examined for ADP expression.

The median age of the primary UM patients at diagnosis was 72.5 years (range 44 - 90). The tumors had a mean largest basal diameter (LBD) of 12.0 mm and a mean thickness of 7.7mm. Seventeen UM (50%) contained epithelioid cells, and 21 cases (61.7%) were classified as monosomy 3 by Multiplex Ligation Dependent Probe Amplification (MLPA), as previously described [5]. ADP was detected in all UM examined, although the proportion of UM cells containing ADP+ LD was variable across the tumor sections analyzed (Figure). High ADP expression (tADP score >4) was documented in 17 specimens (Figure); however, this was not significantly correlated with any clinical, histological or genetic parameters. Notably, normal choroidal melanocytes in the enucleated eyes were ADP negative (not shown). Further, all examined hepatic UM metastases were ADP+, with most showing scores of >4 and with the surrounding liver parenchyma having variable ADP content, according to the underlying state of liver function.

Lipogenesis is essential for cell replication and the upregulation of the lipogenic pathway has become a possible cancer treatment target because of the increased concentrations of intracellular lipids in cancer cells, as a result of aerobic glycolysis (Warburg effect) [6].

This is the first description of ADP protein expression in a small cohort of primary and metastatic UM. Understanding the biological significance of this trait of altered energy metabolism in UM may enable the development of therapies aimed at tumor cell metabolism. Further studies utilizing ADP immunostaining are required in a larger cohort of UM to understand the significance of ADP expression in these tumors, and the ability of the UM cells to survive during the metastatic process.

**Patient Consent:**

All patients have consented to the submission of the Letter to the journal.

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**Figure legend:**

(A): Lipid droplets in UM cell culture demonstrated using the Oil-Red-O stain; (B) H&E staining of a UM with mixed cell morphology (objective x20) and (C) a weak tADP score with only scattered UM cells with ADP positivity (arrow) (objective x20); (D) H&E staining of another UM with foamy-like cytoplasm of the tumor cells, which were predominantly epithelioid (objective x20); (E) corresponding strong ADP staining of the same UM (objective x20); (F) ADP staining at higher power (objective x40), demonstrating the lipids within the melanoma cells, in some showing a perinuclear pattern (arrows).