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Calcium regulation by temperature-sensitive transient receptor potential channels in human uveal melanoma cells☆,☆☆,★,★★

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ABSTRACT

Uveal melanoma (UM) is both the most common and fatal intraocular cancer among adults worldwide. As with all types of neoplasia, changes in Ca^{2+} channel regulation can contribute to the onset and progression of this pathological condition. Transient receptor potential channels (TRPs) and cannabinoid receptor type 1 (CB1) are two different types of Ca^{2+} permeation pathways that can be dysregulated during neoplasia. We determined in malignant human UM and healthy uvea and four different UM cell lines whether there is gene and functional expression of TRP subtypes and CB1 since they could serve as drug targets to either prevent or inhibit initiation and progression of UM. RT-PCR, Ca^{2+} transients, immunohistochemistry and planar patch-clamp analysis probed for their gene expression and functional activity, respectively. In UM cells, TRPV1 and TRPM8 gene expression was identified. Capsaicin (CAP), menthol or icilin induced Ca^{2+} transients as well as changes in ion current behavior characteristic of TRPV1 and TRPM8 expression. Such effects were blocked with either La^{3+}, capsazepine (CPZ) or BCTC. TRPA1 and CB1 are highly expressed in human uvea, but TRPA1 is not expressed in all UM cell lines. In UM cells, the CB1 agonist, WIN 55,212-2, induced Ca^{2+} transients, which were suppressed by La^{3+} and CPZ whereas CAP-induced Ca^{2+} transients could also be suppressed by CB1 activation. Identification of functional TRPV1, TRPM8, TRPA1 and CB1 expression in these tissues may provide novel drug targets for treatment of this aggressive neoplastic disease.

1. Introduction

Uveal melanoma (UM) is a sight threatening disease in which patient survival rates are poor once this tumor metastasizes out of the eye into the liver, lung, bone and skin. It is the second most prevalent malignant tumor of melanocytes annually affecting 5.1 million people [1,2]. UM is very insidious since it can at first develop asymptomatically in either the posterior uvea (choroid layer) or the anterior uvea (iris and ciliary body) before causing visual loss [3]. Tumor progression can lead to rupture of Bruch's membrane resulting in a typical mushroom-shape configuration. Over the past few decades, UM detection and treatment conditions have considerably improved. However, the overall mortality rate for UM remains high pointing to the need for further studies on the underlying mechanisms of this devastating disease.

Despite the uncertainties about the pathophysiological mechanisms underlying UM progression, there is some indication that transient receptor channel (TRP) functional expression may be involved since this channel superfamily was described in diverse types of tumors [4,5]. Moreover thermosensitive TRP channel expression may be associated with increases in vascular endothelial growth factor (VEGF) expression and micrometastases that were identified in an UM model [6]. Such increases in VEGF described in this model are relevant since its secretion is increased by the same elevated temperature that also activates heat-sensitive TRPs (vanilloid subfamily) [7]. Moreover, this potent angiogenic factor is highly expressed in the aqueous humor and/or vitreous humor of UM patients and in RPE cells. This possible association between heat-sensitive TRPV channel expression and VEGF secretion prompted us

Abbreviations: TRPV, transient receptor potential vanilloid; TRPM, transient receptor potential melastatin; CB1, cannabinoid receptor 1; CAP, capsaicin; CPZ, capsazepine; La^{3+}, lanthanum chloride; BCTC, N-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl) tetrahydropyrazine-1(2H)-carboxamide; WIN, WIN 55,212-2.

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to probe for functional TRP channel expression in some types of normal uveal tissue, four different UM cell lines and one RPE cell line.

TRPs constitute a superfamily of 26 different genes that are subdivided into 7 different subfamilies. The vanilloid subfamily members are designated as TRPV1-7. This group has both heat and cold sensitive members. TRPV1-4 are heat activated whereas some other melastatin and ankyrin subfamily members are cold receptors: TRPM8 (menthol receptor) and TRPA1 [8,9]. As alluded to above, TRPM8 is highly expressed in tumors of the prostate, breast, colon, lung and skin, while TRPV6 is upregulated in prostate, breast, thyroid, colon and ovarian carcinomas [10,11]. On the other hand, TRPM1 expression declines with increases in the degree of melanoma aggressiveness [12,13]. Lastly, in etoposide-resistant retinoblastoma cells, TRPA1 channels are absent suggesting a role in providing sensitivity to cytotoxicants [14]. There is ample evidence for the functional involvement of TRP vanilloid receptor 1 (TRPV1) (capsaicin receptor) in cancer. It is highly expressed in cancers of the prostate, colon, urothelium and pancreatic neuroendocrine tumors [15–18]. Moreover, TRPV1 expression has been correlated with the aggressiveness of some types of cancer progression [19,20]. Therefore, drug targeting TRP function to suppress its activation may provide a novel therapeutic option to treat some types of cancer.

One approach to suppress TRPV1 activation may involve stimulating cannabinoid (CB) receptors since in a murine corneal wound healing model TRPV1-induced scarring and inflammation were more severe in CB1 knockout mice than in their wildtype counterpart [21,22]. This improved wound healing outcome is relevant since it suggests that CB1 activation in wildtype mice blunts TRPV1 activation. One such potent endogenous cannabinoid released by injury in the cornea is

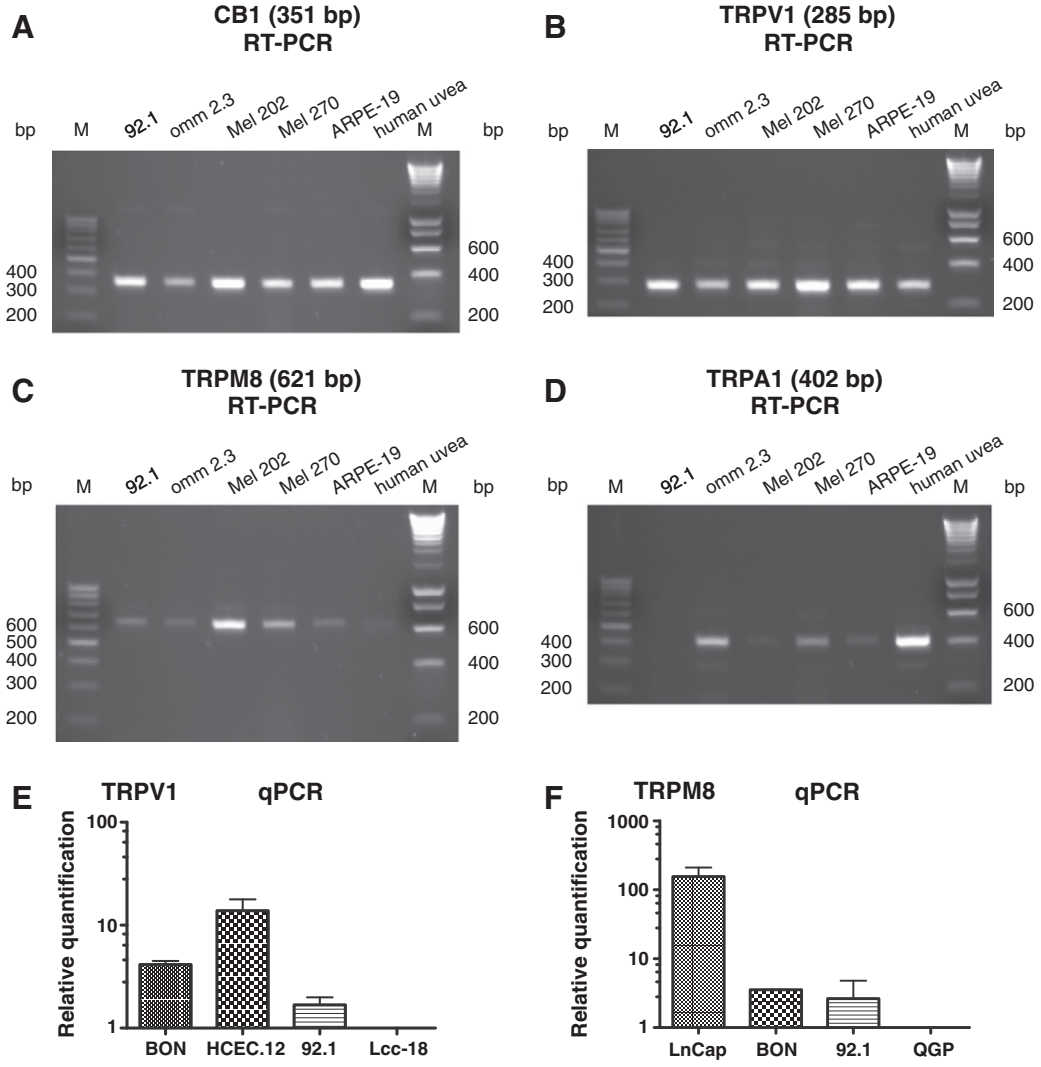

Fig. 1. CB1, TRPV1, TRPM8, and TRPA1 gene expression in human UM cell lines and healthy human uvea. (A) RT-PCR side-by-side ran under the same conditions for the UM cell lines 92.1, omm 2.3, Mel 202, Mel 270, the human retinal pigment epithelial (RPE) cell line ARPE-19 and human healthy uvea. CB1 (351 bp) expression could be detected in all UM cell lines and ARPE-19 as well as human uvea. M = DNA marker. (B) TRPV1 (285 bp) expression could also be detected in all UM cell lines, ARPE-19 and the human uvea. (C) TRPM8 (621 bp) expression could also be detected in all UM cell lines and ARPE-19 investigated. (D) TRPA1 (402 bp) expression could be detected in normal human uvea. Similar to the TRPM8 mRNA signals, there are different TRPA1 mRNA signals regarding the UM cell lines and ARPE-19. TRPA1 expression could not be detected in the 92.1 UM cell line. (E) Quantitative gene expression study of mRNA signals of TRPV1, in BON cells (pancreatic neuroendocrine tumor), Lcc-18 cells (human colon neuroendocrine tumor), human corneal endothelial cells (HCEC-12) and the 92.1 UM cell line using quantitative real-time RT-PCR. The bar chart shows that 92.1 cells express TRPV1. The data were normalized to the Lcc-18 cell line with the lowest mRNA signals. (F) Quantitative gene expression study of mRNA signals of TRPM8, in BON cells, QGP cells (human neuroendocrine tumor), human prostate carcinoma cells (LNCaP) and the 92.1 UM cell line using quantitative real-time RT-PCR. The data were normalized to the QGP cell line with the lowest mRNA signals.