ASIC1/2, TRPV1/4 in skin Tumors

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The acid-sensing ion channels ASIC1 and ASIC2, as well as the transient receptor potential vanilloid channels TRPV1 and TRPV4, are proton-gated cation channels that can be activated by low extracellular pH (pHe), which is a hallmark of the tumor microenvironment in solid tumors. However, the role of these channels in the development of skin tumors is still unclear.

Keywords: melanoma ; squamous cell carcinoma ; basal cell carcinoma ; proton-sensitive ion channels

1. Introduction

Melanoma and non-melanoma skin cancers (NMSCs) are the most prevalent cancers among the white population, exhibiting an increasing incidence rate worldwide [1]. The WHO counts between 2 to 3 million new cases of NMSC per year, being 18–20 times higher than melanoma. However, due to its risk of metastasis, the malignant melanoma (MM) is responsible for 90% of deaths among skin cancers, with a yearly increasing incidence rate between 4 and 6% [2]. The group of NMSC includes basal cell carcinomas (BCCs), which account for around 80% of NMSC, and squamous cell carcinomas (SCCs), with around 20% of NMSC. Only 1% can be classified as other skin tumors [3]. Nevus cell nevi (NCN) are benign neoplasms, but about 10–30% of melanomas arise from NCN [4]. Even if the mortality rate and metastatic potential of NMSCs are low, those tumors lead to enormous morbidity and extensive costs for our health system [5]. Therefore, it is important to find new therapeutic targets in MM and NMSC for future treatments.

Tumor formation changes the physical microenvironment in the tissue. Little vascular perfusion, regional hypoxia and the subsequent anaerobic glucose metabolism lead to lactic acid and, hence, to extracellular acidosis in tumors with extracellular pH (pH_e) as low as 6.5 [6]. Furthermore, membrane-bound transporters (monocarboxylate transporters MCTs 1–4, carboanhydrases CA2/9/12, sodium hydrogen exchanger 1 NHE, vacuolar type ATPases VATPases, sodium bicarbonate symporters) contribute to the acidified tumor microenvironment (TME) [7]. In physiological conditions, the pH_e is higher (7.2–7.4) than the intracellular pH_i (6.9–7.2), whereas in a tumor environment, the so-called reversed pH gradient ($pH_e < pH_i$) develops [8]. This reversed pH gradient (or inside-out pH gradient) is harmful to normal cells, as cellular acidification in general leads to apoptosis. In tumor cells, however, it causes migration and invasion and, hence, benefits tumor growth [6]. In contrast to normal cells, tumor cells can adjust to survive in low pH by increasing glycolytic activity and expression of proton transporters, which stabilize intracellular pH [9]. Several of these transporters and pumps have already been detected to play a role in the maintenance of TME, such as carbonic anhydrases (CA2,CA9, CA12), V-ATPases (vacuolar-type H^+ ATPases), Na^+/HCO^- 3-Co-transporters, the monocarboxylate transporters MCT 1–4 or Na^+/H^+ exchanger 1 (NHE1) [10]. Through changes in their expression or activity, these plasma membrane proteins promote H^+ efflux, thus leading to the typical alkaline pH_i and the acidic pH_e in tumor cells [10]. Cancer cells need to detect the dysregulated pH by sensors to mediate adequate cellular response. Acid-sensing proteins transmit signals to the cytoplasm and nucleus, hence influencing intracellular signal transduction pathways and gene expression [10]. One group of these sensors is the proton-sensitive G-protein coupled receptors (pH-GPCRs) [11]. We recently published first data on the expression profiles of pH-GPCRs in various skin tumors [8][12].

Other proton-sensing sensors in the plasma membrane are the transient receptor potential vanilloid channels (TRPVs) as well as the acid-sensitive ion channels (ASICs). Little is, however, known on their expression and role in skin tumors.

Transient receptor vanilloid potential ion channels (TRPVs) are a group of subfamilies numerously and diversely expressed in several tissues and organs, where they perform pleiotropic physiological and pathological functions. These nonselective cation channels were originally characterized as “polymodal cellular sensors” in neurons, being activated by chemical, physical and thermal stimuli [13]. A subgroup of these channels are the Ca^{2+}-permeable, nonselective thermo-TRPs TRPV1 and TRPV4 [14]. These proton-sensing proteins are both activated by extracellular acidity [10]. Furthermore, TRPV1 is stimulated by vanilloid compounds (capsaicin and resiniferatoxin), injurious heat (≥43 °C) and some eicosanoids [15]. TRPV4 is activated by lower temperature (>24 °C) and by hypoosmotic stimulation [15]. Apart from

neuronal cells, the expression of TRPV1 and TRPV4 has been proven in a wide range of tissues, amongst others in epidermal keratinocytes [16]. Moreover, they play a role in the regulation of cell apoptosis and survival by regulating calcium signaling, which is essential for the apoptosis-driven differentiation program of keratinocytes [16]. TRPV1 has been found within the skin in epidermal and hair follicle keratinocytes, dermal mast cells, in sebaceous glands and dendritic cells [15]. TRPV4 contributes additionally to cell survival after skin exposure to heat and to the control of skin permeability barrier by modulating tight junction proteins [17]. Its activation promotes barrier regeneration, which was demonstrated by the fact that an inferior epidermal barrier (e.g., untight cell–cell junctions) was found in TRPV4-deficient mice [13]. TRPV4 has been identified in basal and suprabasal keratinocytes [15].

Acid-sensing ion channels (ASICs) are cation channels that belong to the degenerin/epithelial Na^+ channel (DEG/ENaC) superfamily and can be activated by extracellular acidification. They are mostly expressed in the central nervous system and in peripheral sensory neurons. There are seven subunits from four genes (namely ASIC1a, ASIC1b1, ASIC1b2, ASIC2a, ASIC2b, ASIC3 and ASIC4) [10]. Each subunit comprises two transmembrane domains, connected with a large extracellular cysteine-rich loop, which are trimeric assemblies. ASIC1 is Na^+- and Ca^{2+}-permeable, whereas other types of ASICs are only permeable to Na^+ [6]. ASICs have different functions in the peripheral and central nervous system in physiological as well as in pathological processes. In the CNS, ASIC1 channels participate in neuroplasticity, regulation of fear behaviors, learning, memory functions and pain sensation [18]. ASIC2 plays a role in retinal integrity and neuronal viability in cerebral ischemia [19]. In the peripheral nervous system, they are involved in nociception and mechano-sensation [20]. More relevant for the current study, however, is the fact that these ASICs are also expressed in non-neuronal cells (e.g., keratinocytes, bone, dendritic cells, vascular smooth muscle [6]), where they contribute to pH homeostasis, cellular migration and inflammation [21].

There are a few reports about the expression and the functions of TRPVs and ASICs in other tumors [22]. Nevertheless, there is no sufficient information about their presence and function in skin tumors. In this study, we investigate the expressions of ASIC1, ASIC2, TRPV1 and TRPV4 in squamous cell carcinoma (SCC), basal cell carcinoma (BCC), malignant melanoma (MM) and in nevus cell nevi (NCN).

2. ASIC1

Concerning the tumor tissues investigated in this study, ASIC1 is strongly expressed in SCC, BCC and in NCN in both epidermal and dermal portions. Epidermal and dermal MM varied in expression levels. Even though in the literature there is little information about the expression of ASIC1 in melanomas and NMSC, the role in cancer progression has been proven in other tissues. In malignant glioma ASIC1 plays a role in the growth and migration of the tumor cells [23]. Gupta et al. detected that ASIC1 contributes to breast cancer pathogenesis and that ASIC1 inhibitors lead to a significant reduction in tumor growth in mice [24]. Even in human lung adenocarcinoma cells (cell line A549) ASIC1s might be a prognostic marker [6]. Taking these considerations and our results together, ASIC1 might serve as a potential therapeutic target, but further functional studies are required to fully understand the role of ASIC1 in tumor progression.

3. ASIC2

ASIC2 shows a negative expression profile in BCC, whereas the dermal portion of NCN is strongly expressed. These inhomogeneous results mirror previous knowledge concerning ASIC2 in other tumors. ASIC2 being less expressed is consistent with findings by Berdiev et al., who investigated ASIC2 in malignant gliomas [23]. The authors found that ASIC2 is not expressed in the plasma membrane of glial cells, whereas ASIC1 is indeed expressed on these tumor cells, analogous to our findings in BCC. According to them, ASIC1 and ASIC2 are co-expressed in normal cells, and the lack of ASIC2 in tumor cells leads to a large inward cation current. Inhibiting this current reduces glioma growth and cell migration [25]. It remains to be investigated if these voltage-independent cation currents present in gliomas are also existent in BCC, making the inhibition of this conductance a potential therapeutic target. Our results regarding the positive expression of ASIC2 in dermal NCN are in accordance with findings by Zhou et al. They detected an up-regulation of ASIC2 in colorectal cancer, leading to increased cell proliferation, whereas a knockdown had the opposite effect [26].

4. TRPV1

In all of our investigated tumors, expression of TRPV1 was high. As mentioned before, TRPV1 is associated with the processes of inflammation and calcium signaling [16]. As both chronic inflammation as well as abnormal calcium signaling play a role in tumorigenesis, it seems plausible that TRPV1 is involved in tumor progression. Marincsák et al. detected a drastic elevated expression of TRPV1 in SCC of the human tongue and in precancerous lesions [27]. Additionally, in other head-and-neck SCC localized on the oral floor or the gingiva, TRPV1 expression is upregulated [28]. To investigate the

effect of TRPV1 antagonists on skin tumor formation, Park et al. treated TRPV1 in keratinocytes with competitive antagonists (AMG-9810 and SB-705498) to potentially use TRPV1 as a pharmacological target, but they could not find skin tumor promotion in epidermal keratinocytes treated by the antagonists [29]. Research linking TRPV1 to carcinogenesis treatment needs to be further conducted, as the evidence from the literature seems controversial so far. Thus, even if we could show that TRPV1 shows a higher expression level in all our investigated tumors, further studies need to be conducted to better understand the exact role of TRPV1 in skin tumor formation to use it as a potential therapeutic target.

5. TRPV4

TRPV4 is overexpressed in SCC, and in BCC it also shows a positive expression profile. In MM it shows mixed reactions, and in the dermal portion of NCN negative expression is predominant. Previous studies reported that TRPV4 was involved in tumorigenesis in different kinds of cancers, such as in esophageal squamous cell carcinoma, where we can see an upregulation of TRPV4 [30]. Huang et al. activated TRPV4 in esophageal SCC, which resulted in cellular migration of the tumor cells [30]. Additionally, in gastric cancer TRPV4 is upregulated and is even associated with higher tumor invasion, lymph node metastasis and poor survival [31]. Contradictory to our results, another research group detected a downregulation of TRPV4 in specific NMSC, as Bowen's disease (BD), solar keratosis (SK), and also BCC and SCC [15]. We cannot support these findings, as our stainings deliver positive and even higher expression of TRPV4 in SCC and BCC compared to normal keratinocytes. Based on this, it is plausible to speculate that the expression of TRPV4 (as well as the other channels) varies between patients, types and subtypes of cancer and micro- and macroenvironments.

In conclusion, ASIC1, ASIC2, TRPV1 and TRPV4 are expressed by most common skin tumors. However, there are some interesting expression patterns and differences, as noted above. Our findings need to be reinforced by a larger sample size, RNA expression analysis (e.g., RNAScope) and by functional studies that investigate the precise roles of the ion channels in tumor formation. This could potentially lead to drugs that target the investigated ion channels in order to manipulate the TMA and/or the cellular response towards the inside-out pH gradient in solid skin cancers.

References

1. Apalla, Z.; Nashan, D.; Weller, R.B.; Castellsagué, X. Skin Cancer: Epidemiology, Disease Burden, Pathophysiology, Diagnosis, and Therapeutic Approaches. Dermatol. Ther. 2017, 7, 5–19.
2. Ward, W.H.; Farma, J.M. (Eds.) Contributors. In Cutaneous Melanoma: Etiology and Therapy; Codon Publications: Singapore, 2017; pp. xi–xiv.
3. Eisemann, N.; Waldmann, A.; Geller, A.C.; Weinstock, M.A.; Volkmer, B.; Greinert, R.; Breitbart, E.W.; Katalinic, A. Non-Melanoma Skin Cancer Incidence and Impact of Skin Cancer Screening on Incidence. J. Investig. Dermatol. 2014, 134, 43–50.
4. E Damsky, W.; Bosenberg, M. Melanocytic nevi and melanoma: Unraveling a complex relationship. Oncogene 2017, 36, 5771–5792.
5. Apalla, Z.; Lallas, A.; Sotiriou, E.; Lazaridou, E.; Ioannides, D. Epidemiological trends in skin cancer. Dermatol. Pract. Concept. 2017, 7, 1–6.
6. Wu, Y.; Gao, B.; Xiong, Q.-J.; Wang, Y.-C.; Huang, D.-K.; Wu, W.-N. Acid-sensing ion channels contribute to the effect of extracellular acidosis on proliferation and migration of A549 cells. Tumor Biol. 2017, 39.
7. Neri, D.; Supuran, C.T. Interfering with pH regulation in tumours as a therapeutic strategy. Nat. Rev. Drug Discov. 2011, 10, 767–777.
8. Nassios, A.; Wallner, S.; Haferkamp, S.; Klingelhöffer, C.; Brochhausen, C.; Schreml, S. Expression of proton-sensing G-protein-coupled receptors in selected skin tumors. Exp. Dermatol. 2018, 28, 66–71.
9. Rohani, N.; Hao, L.; Alexis, M.S.; Joughin, B.A.; Krismer, K.; Moufarrej, M.N.; Soltis, A.R.; Lauffenburger, D.A.; Yaffe, M.B.; Burge, C.B.; et al. Acidification of Tumor at Stromal Boundaries Drives Transcriptome Alterations Associated with Aggressive Phenotypes. Cancer Res. 2019, 79, 1952–1966.
10. Edamaghi, M.; Wojtkowiak, J.W.; Gillies, R.J. pH sensing and regulation in cancer. Front. Physiol. 2013, 4, 370.
11. Weiß, K.T.; Fante, M.; Köhl, G.; Schreml, J.; Haubner, F.; Kreutz, M.; Haverkampf, S.; Berneburg, M.; Schreml, S. Proton-sensing G protein-coupled receptors as regulators of cell proliferation and migration during tumor growth and wound healing. Exp. Dermatol. 2017, 26, 127–132.

12. Klatt, W.; Wallner, S.; Brochhausen, C.; Stolwijk, J.A.; Schreml, S. Expression profiles of proton-sensing G-protein coupled receptors in common skin tumors. Sci. Rep. 2020, 10, 1–12.
13. Tóth, B.I.; Oláh, A.; Szöllősi, A.G.; Bíró, T. TRP channels in the skin. Br. J. Pharmacol. 2014, 171, 2568–2581.
14. Nilius, B.; Vriens, J.; Prenen, J.; Droogmans, G.; Voets, T. TRPV4 calcium entry channel: A paradigm for gating diversity. Am. J. Physiol. Physiol. 2004, 286, C195–C205.
15. Fusi, C.; Materazzi, S.; Minocci, D.; Maio, V.; Oranges, T.; Massi, D.; Nassini, R. Transient Receptor Potential Vanilloid 4 (TRPV4) Is Downregulated in Keratinocytes in Human Non-Melanoma Skin Cancer. J. Investig. Dermatol. 2014, 134, 2408–2417.
16. Bujak, J.K.; Kosmala, D.; Szopa, I.M.; Majchrzak, K.; Bednarczyk, P. Inflammation, Cancer and Immunity—Implication of TRPV1 Channel. Front. Oncol. 2019, 9, 1087.
17. Lee, W.H.; Choong, L.Y.; Mon, N.N.; Lu, S.; Lin, Q.; Pang, B.; Yan, B.; Krishna, V.S.R.; Singh, H.; Tan, T.Z.; et al. TRPV4 Regulates Breast Cancer Cell Extravasation, Stiffness and Actin Cortex. Sci. Rep. 2016, 6, 27903.
18. Huang, Y.; Jiang, N.; Li, J.; Ji, Y.-H.; Xiong, Z.-G.; Zha, X.-M. Two aspects of ASIC function: Synaptic plasticity and neuronal injury. Neuropharmacology 2015, 94, 42–48.
19. Boscardin, E.; Alijevic, O.; Hummler, E.; Frateschi, S.; Kellenberger, S. The function and regulation of acid-sensing ion channels (ASICs) and the epithelial Na+channel (ENaC): IUPHAR Review 19. Br. J. Pharmacol. 2016, 173, 2671–2701.
20. Sherwood, T.W.; Frey, E.N.; Askwith, C.C. Structure and activity of the acid-sensing ion channels. Am. J. Physiol. Physiol. 2012, 303, C699–C710.
21. Gu, Q.; Lee, L.-Y. Acid-Sensing Ion Channels and Pain. Pharmaceuticals 2010, 3, 1411–1425.
22. Santoni, G.; Caprodossi, S.; Farfariello, V.; Liberati, S.; Gismondi, A.; Amantini, C. Antioncogenic Effects of Transient Receptor Potential Vanilloid 1 in the Progression of Transitional Urothelial Cancer of Human Bladder. ISRN Urol. 2012, 2012, 1–11.
23. Berdiev, B.K.; Xia, J.; McLean, L.A.; Markert, J.M.; Gillespie, G.Y.; Mapstone, T.B.; Naren, A.P.; Jovov, B.; Bubien, J.K.; Ji, H.-L.; et al. Acid-sensing Ion Channels in Malignant Gliomas. J. Biol. Chem. 2003, 278, 15023–15034.
24. Gupta, S.C.; Singh, R.; Asters, M.; Liu, J.; Zhang, X.; Pabbidi, M.R.; Watabe, K.; Mo, Y.-Y. Regulation of breast tumorigenesis through acid sensors. Oncogene 2016, 35, 4102–4111.
25. Vila-Carriles, W.H.; Kovacs, G.G.; Jovov, B.; Zhou, Z.-H.; Pahwa, A.K.; Colby, G.; Esimai, O.; Gillespie, G.Y.; Mapstone, T.B.; Markert, J.M.; et al. Surface Expression of ASIC2 Inhibits the Amiloride-sensitive Current and Migration of Glioma Cells. J. Biol. Chem. 2006, 281, 19220–19232.
26. Zhou, Z.-H.; Song, J.-W.; Li, W.; Liu, X.; Cao, L.; Wan, L.-M.; Tan, Y.-X.; Ji, S.-P.; Liang, Y.-M.; Gong, F. The acid-sensing ion channel, ASIC2, promotes invasion and metastasis of colorectal cancer under acidosis by activating the calcineurin/NFAT1 axis. J. Exp. Clin. Cancer Res. 2017, 36, 1–12.
27. Marincsák, R.; I Tóth, B.; Czifra, G.; Márton, I.; Redl, P.; Tar, I.; Tóth, L.; Kovács, L.; Bíró, T. Increased expression of TRPV1 in squamous cell carcinoma of the human tongue. Oral Dis. 2009, 15, 328–335.
28. Kiss, F.; Pohóczky, K.; Szállási, A.; Helyes, Z. Transient Receptor Potential (TRP) Channels in Head-and-Neck Squamous Cell Carcinomas: Diagnostic, Prognostic, and Therapeutic Potentials. Int. J. Mol. Sci. 2020, 21, 6374.
29. Park, M.; Naidoo, A.A.; Burns, A.; Choi, J.K.; Gatfield, K.M.; Vidgeon-Hart, M.; Bae, I.-H.; Lee, C.S.; Choi, G.; Powell, A.; et al. Do TRPV1 antagonists increase the risk for skin tumourigenesis? A collaborative in vitro and in vivo assessment. Cell Biol. Toxicol. 2018, 34, 143–162.
30. Huang, R.; Wang, F.; Yang, Y.; Ma, W.; Lin, Z.; Cheng, N.; Long, Y.; Deng, S.; Li, Z. Recurrent activations of transient receptor potential vanilloid-1 and vanilloid-4 promote cellular proliferation and migration in esophageal squamous cell carcinoma cells. FEBS Open Bio 2018, 9, 206–225.
31. Wang, H.; Zhang, B.; Wang, X.; Mao, J.; Li, W.; Sun, Y.; Yuan, Y.; Ben, Q.; Hua, L.; Qian, A. TRPV4 Overexpression Promotes Metastasis Through Epithelial–Mesenchymal Transition in Gastric Cancer and Correlates with Poor Prognosis. Onco Targets Ther. 2020, 13, 8383–8394.