Can capsaicin present in food act as carcinogenic, antitumor or both

Guilherme Barroso Langoni de Freitas^{1, 2, *}, Najeh Maissar Khalil², Iara José de Messias-Reason¹

¹Post-Degree Program in Internal Medicine and Health Sciences at UFPR, Department of Clinical Patology, Federal University of Parana, Curitiba, Parana, Brazil

²Department of Pharmacy, State University of Center-West, Guarapuava, Parana, Brazil

Email address:

prof.gbarroso@yahoo.com.br (G. B. L. de Freitas), najehunicentro@hotmail.com (N. M. Khalil),

iaramessias@yahoo.com.br (I. J. de Messias-Reason)

To cite this article:

Guilherme Barroso Langoni de Freitas, Najeh Maissar Khalil, Iara José de Messias-Reason. Can Capsaicin Present in Food Act as Carcinogenic, Antitumor or Both. *Cancer Research Journal*. Special Issue: Lifestyle and Cancer Risk. Vol. 2, No. 6-1, 2014, pp. 34-41. doi: 10.11648/j.crj.s.2014020601.14

Abstract: Pepper is amongst the most widely consumed spices in the world. However, what few people know, is that the pungent substance responsible for its blazing characteristic has many other biological properties, e.g. analgesic, antiinflammatory, antitumor and even carcinogenic. Several studies have discussed the antitumor and carcinogenic potential of this secondary metabolite. Nevertheless, the literature still lacks a comprehensive study relating the biological effects of capsaicin with the consumed dose, for both pharmacological and toxicological mechanisms. To solve this deficiency, the aim of this study was to discuss in details all the points mentioned above, in order to clarify the major questions about the subject.

Keywords: Capsaicin, Cancer, Antitumor, Carcinogenic, Mechanism of Action

1. Introduction

Peppers, which belong to the plant genus *Capsicum*, are among the most frequently consumed seasonings through-out the world, mainly in countries with high consume of the same, e.g. India and Mexico. Their principal pungent ingredient is capsaicin (8-methyl-N-vanillyl-6-nonenamide), a phenolic substance with variable and contradictory bio-logical properties. Under determinate physiological conditions, this metabolite can cause antagonistic effects such as neurogenic inflammation or anti-inflammatory and analgesic, or even, antitumor or carcinogenic [1]. To understand these opposite effects, it became necessary to know what are the biological targets and the changes in homeostasis, caused by the capsaicin. These effects are mostly caused by a transient receptor potential vanilloid type, 1 ion channel (TRPV1), which with the cloning in 1997 represented an important step for the understanding of the molecular mechanisms underlying the transduction of thermal and noxious chemical stimuli by the peripheral nociceptors [2-3]. Since then, it became clear that TRPV1 plays a pivotal role in the development of various physiological and pathological processes [3-5]. In addition to

the biological effects generated from the receptor, there are other mediators that should receive special attention, e.g. inflammatory mediators, reactive oxygen species (ROS) and transcription factors. Here, we will discuss about the actual knowledge about capsaicin pharmacology in cancer development and as a therapeutic combat.

2. Activation of TRPV1 by Capsaicin

TRP channels represent a large and diverse family of non-selective cation channels which respond to an ample range of physical and chemical stimuli [6]. Vanilloid channels have an oligomeric structure formed by subunits, which have six transmembrane segments with a pore domain formed by the fifth and sixth transmembrane regions and by the intracellular N- and C-termini [7]. Among the six TRPV members (TRPV1-6), the vanilloid receptor subtype-1, or TRPV1, has been gaining increased interest as a molecular integrator of physical and chemical noxious stimuli. The TRPV1 receptor can be activate by many different exogenous factors, such as toxins (vanillotoxins) [8], heat (above 42 °C), ions (Na⁺, Mg²⁺ and Ca²⁺) [9], acid and basic pH [10] and membrane depolarization [11].

The expression of the vanilloid TRPV1 receptor is not

restricted to sensory neurons. TRPV1 has also been found in various peripheral non-neuronal tissues of rodents and humans, e.g. kidney [12], gastrointestinal tract [13], urinary tract [14], epidermal keratinocytes [15] and various other tissues [16, 17]. The discovery of non-neuronal tissues expressing the vanilloid TRPV1 receptor suggests that the role of this channel is not limited just to the perception of noxious stimuli [18].

Some techniques are used to solve the three-dimensional structure of biological targets, such as for the TRPV1 receptor, amongst the most commonly used techniques are the: NMR, x-ray crystallography, and recently, the single-particle electron cryomicroscopy (cryo-EM). The main advantage of cryo-EM over X-ray crystallography is that it obtains a three-dimensional structure of the receptor, closer to the native conformation. It occurs because the crystallization is not required in cryo-EM approach [19]. With this technique it became possible to resolve the complete structure of TRPV1 (Fig. 1), PDB codes: 3J5Q [20], 3J5R [21] and 3J5P [21]. TRPV1 channels are homotetramers which the three-dimensional (3D) structure resembles that of voltage-gated ion channels (VGICs). These two channel families share a similar tetrameric architecture in which subunits are arranged in four-fold symmetry around a central ion on the permeation path. Each subunit consists of six transmembrane α -helices (S1–S6) that span the lipid bilayer, plus a re-entrant loop with a pore helix located between S5 and S6, which together assume an ‘inverted teepee’ arrangement, resembling that of VGICs. The ion permeation pathway is formed by the transmembrane segments 5 and 6 (S5 and S6) and by the intervening region of the pore loop (S5–P–S6) [20, 21]. Cao et al., evaluated which conformational changes may occur when two different vanilloid agonists, capsaicin and vanillotoxin (called double-knot toxin DkTx), bind to the TRPV1 receptor. Three models were used to evaluate the conformational changes in the receptor (Apo, Capsaicin and RTX/ DkTx-bound, TRPV1 structures). The binding site is similar between capsaicin and DkTx. Both were found in a hydrophobic membrane-embedded cavity formed by the helices S3 and S4 and the S4 - S5 linker of a subunit, as well as by the helices S5 and S6 of the adjacent subunit [22]. However, when TRPV1 is analyzed from the extracellular side, it becomes possible to identify that the capsaicin binding site sits roughly below the DkTx-active site. Jung et al., localized the pharmacophoric residues (Glu-761 and Arg-114) for capsaicin, in the C- and N-cytosolic tails, respectively [23]. Other important point in the activation of the receptor is that the steric bulk of DkTx causes a larger opening of TRPV1 outer pore when compared to capsaicin. Thus, it was concluded that the capsaicin–TRPV1 complex represents a partially activated state of the receptor and this can be explained by the conformational change of I679 in S6. This residue forms a hydrophobic seal sufficiently narrow (5.3 Å, in Apo state) to block permeation by hydrated ions when TRPV1 is in its closed conformation. Capsaicin and DkTx can alter the position of I679 with consequent expansion of

the lower gate to 7.6 and 9.3 Å, respectively [21].

It justifies the better agonist activity of DkTx when comparing to the capsaicin. Nevertheless, we should high-light that the efficacy and affinity of capsaicin on TRPV1 slightly varies depending on the species and expression system [24].

The activation of TRPV1 results in a non-selective influx of divalent (Ca^{2+} and Mg^{2+}) and monovalent (Na^{+}) cations through the ionic pore on the receptor [25]. In excitable membranes such as neurons, the activation of TRPV1 induces cell depolarization, therefore enabling the generation and propagation of action potentials, such as pain or burning sensations for example. However, the initial effects are followed by a refractory state in which the sensitivity to capsaicin will be inhibited (nociceptor desensitization). Depending on the vanilloid, dose, concentration and administration site, nociceptor refractoriness may last from minutes to months, suggesting the contribution of different cellular mechanisms. So, it can explain the paradoxical use of capsaicin as an analgesic agent to treat different pain disorders [26].

Figure 1. (a) The electrostatic map of TRPV1 receptor shows the electronic differences between the S5-pore-S6 region (hydrophobic, blue surface) and the central pore (negative, red surface). (b) This figure on spacefill allows viewing the homotetramer complex. (c) Bottom view highlighted the central pore (white circle), S5-pore-S6 (also called pore module, yellow circle) and S1-S4 domains seconded by arrows.

It was also observed that capsaicin can lead the local cells to death and induce DNA fragmentation in a concentration-dependent manner [12, 27, 28]. Several

mechanisms can explain this cytotoxic response of the capsaicin, but, first we will introduce the role of TRPV1. The binding of capsaicin to TRPV1 induces an opening of the ion channel, allowing extensive input of Ca^{2+} . The calcium (Ca^{2+}) ion, as a ubiquitous intracellular messenger, regulates many different cellular functions, including contraction, secretion, metabolism, gene expression, cell survival and induction of apoptotic or necrotic cell death, even cell migration (metastasis) [29-31]. Several molecules seem to be involved in the modulation of TRPV1 activity based on the Ca^{2+} influx, e.g. calmodulin (CaM) and ATP. Calmodulin, which binds four Ca^{2+} by Ca^{2+} -CaM complexes, has been involved in the process of Ca^{2+} -dependent desensitization, which reduces responses of TRPV1 on its activators, as on the capsaicin. On the other hand, if ATP is competing for the same binding site as CaM, it can generate an opposite response, i.e. ATP binding prevents desensitization. These observations have proposed that this effect is mediated by modulating the channels sensitivity to calcium fluctuations and possibly to the metabolic state [31].

Calcium is an important regulator of a variety of cellular processes, many of which are dysregulated in cancer cells, such as proliferation, migration and cell death [31, 32]. In several cancer types occurs an increase in the expression of specific calcium permeable ion channels [33] which enables an increase in the intracellular calcium concentration ($[\text{Ca}^{2+}]_i$). In some cases, the Ca^{2+} overload is often associated to the cleavage and inactivation of calcium efflux transporters located on the plasma membrane [34, 35]. The consequence of this attenuation of the Ca^{2+} efflux mechanism is the elevation in $[\text{Ca}^{2+}]_i$ and promotion of cell death pathways modulated by Ca^{2+} , such as those of the mitochondria [35]. These observations explain the apoptotic response overexpression of TRPV1 after capsaicin activation lung [36], glioma [37], urothelial [38] and KB cells (epidermal carcinoma) [39, 40]. TRPV1 was functionally implicated in these events as they were markedly inhibited by the TRPV1 antagonist, the capsazepine. However, Gonzales *et al.*, investigated the mechanisms of vanilloid cytotoxicity and its anti-tumor effects in oral squamous cell carcinoma (OSCC) and showed that OSCC cell lines treated with capsaicin had significantly reduced cell viability, but pre-treatment with capsazepine failed to reverse these effects. Moreover, capsazepine alone was significantly cytotoxic to tumor cells, suggesting that the mechanism of action is independent of TRPV1 activation, which was confirmed by calcium imaging, indicating that TRPV1 channels were not functional in the tested cell lines. In order to examine whether the observed cytotoxicity was due to the generation of ROS and subsequent apoptosis, it was used flow cytometry and co-treatment with the antioxidant N-acetyl-cysteine (NAC). The two analyses confirmed the hypothesis, namely the apoptosis was produced by ROS induction [39].

Despite some studies underestimated the role of the TRPV1 receptor in cancer cell development, evidences have shown an important function on cancer control. Tests in 5637

cells-lacking TRPV1 receptor that were treated with capsaicin showed upregulation of pro-angiogenic (angiopoietin 1, angiopoietin 2 and vascular endothelial growth factor), pro-invasive and pro-metastatic genes (MMP1, MMP9, TIMP1, TIMP3, granzyme A (GZMA), NM23A and S100A) with a down-regulation of apoptotic genes (Fas/CD95 and tumor necrosis factor receptor, superfamily member 1A). In order to evaluate the relation between lack of TRPV1 expression and increased capsaicin-induced invasiveness, Caprodossi *et al* transfected 5637 cells with the TRPV1 complementary DNA (cDNA) sequence. TRPV1-expressing cells showed increase in calcium level, growth inhibition and apoptosis. Moreover, the cell migration and MMP9 activation were reverted, suggesting an inhibitory of TRPV1 in the urothelial cancer cell invasion and metastasis [40].

In spite of some clinical observations about the receptor, there is a question that still needs to be answered. Is there a dose-dependent relation between capsaicin and its effect? Trying to answer this question, Sanches *et al.*, analyzed the increase in intracellular calcium concentration by different capsaicin concentrations [41]. The vanilloid TRPV1 receptor agonist capsaicin induced a dose-dependent increase in $[\text{Ca}^{2+}]_i$ with apparent full effect occurring at about 1 μM in androgen-sensitive human prostate, adenocarcinoma cells (LNCaP cells). Doses as low as 5 nM of capsaicin were able to increase the $[\text{Ca}^{2+}]_i$ from 22 to 90 nM, whereas 1 μM capsaicin increased it to 190 nM. After this dose of capsaicin, the response was stabilized with a slight decline in the concentration of intracellular calcium, but this effect could be blocked (capsazepine 1 μM inhibit the binding of capsaicin 5 μM) [41]. Other two agonists (resiniferatoxin and (R)-methanandamide), at high doses, were neither able to induce an increase in $[\text{Ca}^{2+}]_i$, suggesting that the mechanism of cell death probably occurs via TRPV1 [42].

What was observed during the refractory period is a pharmacological mechanism generated by persistent stimulus (chemical or physical), which attenuates the nociceptive sensory neuron excitability, making nociceptors partially or totally refractory to subsequent stimuli. It can range from minutes (acute desensitization) and hours (down-regulation) up to days (defunctionalization). Based on its ability to desensitize nociceptors, the irritant vanilloid capsaicin is therapeutically used in the treatment of postherpetic neuralgia [43, 44], diabetic neuropathy [45] and osteoarthritic pain [46]. Through these observations, it was found that prolonged agonist exposure mediates TRPV1 internalization, however the endocytosis pathways are still to be clarified. Another issue that needs to be addressed is the effect of increased $[\text{Ca}^{2+}]_i$, which can induce death and/or promote proliferation and metastasis of cancer cells. The understanding about concentration and exposure time which can induce these effects, may be the answer to the therapeutic and carcinogenic potentials of capsaicin. As mentioned above, the continuous use of capsaicin reduces its sensitivity to the receptor, therefore decreasing the influx of calcium. Nevertheless, it is possible that different cells may also have

different reactions to the exposure of this metabolite. A higher expression of the specific calcium permeable ion channels occurs in a variety of cancer types [33]. In breast cancers, for example, increased levels of the selective calcium channel TRPV6 [47] is associated to poor prognosis, and normally occur the basal subtype [48]. Elevated TRPV1 expression occurs in colon [49], pancreatic [50] and prostate [41, 51] cancer cells. Numerous studies have also now identified that silencing and/or the pharmacological inhibition of overexpressed calcium ion channels in cancer cells may attenuate key aspects of cancer progression, including proliferation and metastasis [52-55].

An alternative approach that has been proposed (but less studied) is the activation of an overexpressed calcium permeable ion channel to induce cancer cell death [55, 56]. Activation of overexpressed ion channels, such as TRPV1 has caused apoptosis of lung cancer cell lines [57]. Calcium is a critical regulator of cell death, with sustained and high ($>1 \mu\text{M}$) increases in intracellular free Ca^{2+} ($[\text{Ca}^{2+}]_i$), associated to the induction of apoptotic or necrotic cell death [29]. The inactivation or cleavage of calcium efflux transporters on plasma membrane is one of the proposals in recent studies to promote cell death by pathways modulated by $[\text{Ca}^{2+}]_i$, such as those of the mitochondria [34, 56, 58]. However, both increase in the transient cytosolic calcium concentration and in $[\text{Ca}^{2+}]_i$ play major role in cell proliferation, growth and division [59]. Moreover, one limitation of activating overexpressed TRPV1 as therapeutic approach against cancer, is the risk of the agonist (e.g. capsaicin) to produce levels of cytosolic calcium concentration, which are not satisfactory to induce cell death, but can support proliferation, metastasis and even induce cancer. It is believed that concentration of agonist and level of calcium channel over-expression may influence the sensitivity of cancer cells to death or to proliferation. Wu et al., investigated these issues using MCF-7 breast cancer cells with inducible TRPV1 over-expression [60]. This study found that concentration of capsaicin and level of TRPV1 expression influence both cell death (by induction of necrotic cell death) and capsaicin-mediated increases of $[\text{Ca}^{2+}]_i$. On the other hand, no cell proliferation was observed by any concentration of capsaicin at any level of receptor expression, including those associated with increases in $[\text{Ca}^{2+}]_i$. In this way, targeting TRPV1 receptor with capsaicin may represent a way to induce death of breast cancer cells without promotion of cellular proliferation. One possible explanation for the observed responses is the c-Fos expression which may be controlled through rapid increases in $[\text{Ca}^{2+}]_i$ [61, 62]. The c-Fos protein can be influenced by several factors resulting in apoptotic or cell proliferation processes [63, 64]. It was observed that pre-neoplastic, lymphoid cell lines [65, 66] and tamoxifen induced cell death in SK-BR-3 breast cancer cells are closely related to a rapid induction of c-Fos expression and consequently cell death [67]. In the study of Wu et al., the peak of c-Fos expression was reported at 3 h after TRPV1 activation with capsaicin and this expression decreased when the cell died (approximately at 6 h). This indicates that c-Fos

expression is a precursor to cell death or simply an early marker of cell death. However, at the same time the excessive influx of calcium causes cell death and can also deregulate some cytosolic homeostasis processes. This intricacy in signaling of calcium means that the dysregulation of the calcium signal can be a feature of certain pathological states, including neurodegenerative diseases and cancer [68-70], or even acts as anti-tumor drug by increasing oxidative stress.

3. Reactive Oxygen Species, Oxidative Stress and Capsaicin

Normal cell functions such as cellular proliferation, gene expression, cell death [71] and defense against infection [72], may be generated by low concentration of reactive oxygen species (ROS) in the cytoplasm. The cellular proliferation occurs by reversible oxidation (direct action of capsaicin) of active sites in transcription factors, such as the nuclear factor kappa B (NF- κ B) and activator protein-1 (AP-1), or indirect induction of mitogen activated protein kinases (MAPKs). ROS and the consequent cellular oxidative stress have long been associated with cancer. This association is rather complex because at the same time that it may induce the disease, excessive oxidative stress may cause apoptosis of tumor cells, noticeable effect of the mechanism of action of many chemotherapeutic agents [73]. Some evidences suggest that ROS positively contribute to proliferation and to other events required for tumor progression [74, 75]. On the other hand, this confers an elevated state of basal oxidative stress in tumor cells, which points toward the necessity of the cells to defend themselves from oxidative damage in order to survive (e.g. over-expression of antioxidant enzymes) [76, 77]. In this regard, it is noted that tumor cells may die by the same systems they require to emerge and develop. The quantitative difference between oxidative stress in cancer and normal cells was related to liver cells [78], leukemia cells [79], breast cells [80] and gastrointestinal cancer [81].

Wu et al. investigated the administration of capsaicin on human esophagus epidermoid carcinoma CE 81T/VGH cells [82]. They observed that capsaicin induced cell cycle arrest and apoptosis through the elevation of intracellular reactive oxygen species. During 2h, CE 81T/VGH cells were treated with different concentrations of capsaicin (0, 50, 100, 150, 200 μM) and the respective ROS productions were analyzed by flow of cytometric methods, which reveal that capsaicin stimulated the activation of caspase-3 in a dose-dependent manner. It is worth mentioning that elevated levels of ROS cause dual response, inducing cell death or DNA damage and genomic instability [83, 84], which may lead to tumorigenesis. In another study conducted by Yang et al, in vitro antitumor activity of capsaicin in colon cancer cells (Colo320DM and LoVo cells – models) was also observed [85]. Capsaicin was shown to induce fragmentation and decrease of DNA contents, as well as phosphatidylserine translocation, which is a characteristic of apoptosis. Once again the apoptosis was

associated with an increase in ROS production and a disorder of the mitochondrial transmembrane potential. Based on the changes described in the mitochondrial membrane and previous conclusions [86], caspase 3 is considered to have a key role in capsaicin-induced apoptosis.

However, there are still contradictory results about the mechanism of action of capsaicin. The apoptotic mechanism may not be only due to caspase 3. Some recent studies demonstrated that cells, which do not express caspase-3 (e.g. MCF-7 cells), induced apoptosis by caspase-independent or non-caspase-3-dependent mechanisms, due to the activation of other effective caspases or the nuclear translocation of the apoptosis-inducing factor (AIF) [87, 88]. Chou *et al.*, also noted that capsaicin induced a reduction of ROS in MCF-7 cells, instead of the increase mentioned by other researches, in which the generation of ROS was believed to be necessary for the antitumor and inhibitory effects of capsaicin [89, 90].

4. Other Possible Mechanisms

Table 1. Toxicity studies of capsaicin using different routes of administration.

Specie	Sex	Via of administration.	DL50 (mg/Kg)
Swiss albino mice	M	Intraperitoneal	7.65
Swiss albino mice	M	Intravenous	0.56
Swiss albino mice	M	Intramuscular	7.80
Swiss albino mice	M	Intragastric	190
Swiss albino mice	M	Subcutaneous	9.00
Swiss albino mice	M	Dermal	> 512
Swiss albino mice	M	Intrarectal	> 218
Rabbit	M, F	Intraperitoneal	> 50
Albino guinea pig	M	Intraperitoneal	1.10
Rat	F	Intraperitoneal	9.50 – 13.20
Syrian golden hamsters	M	Intraperitoneal	> 120
Mouse	M, F	Intraperitoneal	7,65 – 6,50

The LD50 values to acute toxicity of capsaicin have been determinate in some animal species (Table 1). [91]. Observation that capsaicin is a highly toxic compound when the route of administration does not undergo first-pass effects (i.e. injectable routes) seemed to be associated with gastrointestinal enzyme activity which may hydrolyze it. However, high rectal and dermal doses (218 and 512 mg/Kg, respectively) do not cause death or serious toxic signs when administered in mice, characterizing them as routes of low toxicity to capsaicin. Nevertheless, as capsaicin is usually consumed as seasoning and, depending on the culture or country it may be used daily in meals, further studies about subchronic and chronic effects should be performed. One pharmacokinetic step that deserves special attention is the metabolism. Capsaicin becomes more toxic (mutagenic or carcinogenic forms) through metabolism [92], which induces production of an electrophilic epoxide and a subsequent phenoxy radical, or carry out O-demethylation and oxidation to produce semiquinone and quinone derivatives, respectively [92]. It is believed that phenoxy radical, formed from cytochrome P450 E21 (CYP2E1) and carboxyesterases are related to the mutagenicity and carcinogenicity of capsaicin. This electrophilic intermediate, which is reported

to act as suicide inhibitor, can bind covalently with DNA, RNA, proteins and CYP2E1, altering the metabolism of chemical carcinogens [93, 94]. These results suggested that reactive intermediates were produced during catalytic turn over, however, the metabolism of capsaicin by P450 also represents a detoxification mechanism [95]. Therefore, it seems that capsaicin may present opposing activities, on one hand enzymes that assist in the elimination of potential carcinogens, and on the other hand dimerizing with free radicals reducing toxicity.

Although capsaicin might be a potential drug to influence inflammation, the mechanism is still unclear. It is believed that NF- κ B and AP-1, which have been implicated in many inflammatory diseases, were demonstrated to be inhibited by capsaicin

5. Conclusion

Despite capsaicin is being used for long time as seasonings in culinary, there were few studies discussing the possible biological effects on the development and treatment of cancer. Our study concluded that capsaicin has great therapeutic potential as antitumor drug. Its action mechanism can induce cell death through excessive influx of calcium, increase of ROS and inhibition of proliferative transcription factor (NF- κ B and AP-1). Nevertheless, more studies are necessary about the chronic effects on the metabolism, ROS production and influx of calcium in normal cells. Although capsaicin has antioxidant activity, its metabolites (for example phenoxy radical) can bind covalently with key macromolecules, including the CYP2E1 and DNA, thus altering the metabolism of chemical carcinogens and causing damage to DNA, respectively. The antitumor potential of capsaicin should be explored in an acute form, in order to reduce the risk of damaging normal cells. The development of new drug forms, such as nanoparticles, could increase the specificity of capsaicin on tumor cells, reducing the toxic risk of the substance.

References

- [1] Joon Surh Y (2002) More Than Spice: Capsaicin in Hot Chili Peppers Makes Tumor Cells Commit Suicide. *J the Natl Cancer Inst* 94 (17), 1263-1265.
- [2] Caterina M J (1997) The Capsaicin Receptor: A Heat-Activated Ion Channel in the Pain Pathway. *Nature* 389, 816-24.
- [3] Messeguer A, Planells-Cases R, Ferrer-Montie A (2006) Physiology and Pharmacology of the Vanilloid Receptor. *Curr Neuropharmacol* 4 (1), 1–15.
- [4] Walder R Y, Radhakrishnan R, Loo L, Rasmussen L A, Mohapatra D P, Wilson S P, Sluka K A (2012) TRPV1 is Important for Mechanical and Heat Sensitivity in Uninjured Animals and Development of Heat Hypersensitivity After Muscle Inflammation. *Pain* 53 (8), 1664-72.
- [5] Ho K W, Ward N J, Calkins D J (2012) TRPV1: A Stress Response Protein in the Central Nervous System. *Am J Neurodegener Dis* 1 (1), 1–14.

- [6] Venkatachalam K, Montell C (2007) TRP Channels. *Annu Rev Biochem* 76, 387–417.
- [7] Ferrer-Montiel C, et al (2004) Molecular Architecture of the Vanilloid Receptor. Insights for Drug Design. *Eur J Biochem*, 271(10), 1820-1826.
- [8] Min J W, Liu W H, He X H, Peng B W (2013) Different Types of Toxins Targeting TRPV1 in Pain. *Toxicon*. 71, 66-75.
- [9] Szolcsányi J. (2014) Capsaicin and Sensory Neurones: a Historical Perspective. *Prog Drug Res*. 68, 1-37.
- [10] Dhaka A, Uzzell V, Dubin A E, Mathur J, Petrus M, Bandell M, Patapoutian A (2009) TRPV1 is Activated by Both Acidic and Basic pH. *J Neurosci*, 29(1), 153–158.
- [11] Baumann T K, Chaudhary P, Martenson M E (2004) Background Potassium Channel Block and TRPV1 Activation Contribute to Proton Depolarization of Sensory Neurons from Humans with Neuropathic Pain. *Eur J Neurosci* 19 (5), 1343-1351.
- [12] Sanchez J F, Krause J E, Cortright D E (2001) The Distribution and Regulation of Vanilloid Receptor VR1 and VR1 5'Splice Variant RNA Expression in Rat *Neurosci* 107, 373-381.
- [13] Ward S M, Bayguinov J, Won K J, Grundy D, Berthoud H R (2003) Distribution of the Vanilloid Receptor (VR1) in the Gastrointestinal Tract. *J Comp Neurol* 465, 121–135.
- [14] D. Ost, T. Roskams, F. Van Der Aa, D. De Ridder Topography of the Vanilloid Receptor in the Human Bladder: More Than Just the Nerve Fibers *J. Urol.*, 168 (2002), pp. 293–297
- [15] Stander S, et al (2004) Expression of Vanilloid Receptor Subtype 1 in Cutaneous Sensory Nerve Fibers, Mast Cells, and Epithelial Cells of Appendage Structures. *Exp Dermatol* 13, 129–139.
- [16] Veronesi B, Oortgiesen M (2006) The TRPV1 Receptor: Target of Toxicants and Therapeutics. *Toxicol Sci* 89 (1), 1-3.
- [17] Birder L A, et al (2001) Vanilloid Receptor Expression Suggests a Sensory Role for Urinary Bladder Epithelial. *Cells Proc Natl Acad Sci USA* 98 (23), 13396-401.
- [18] Caterina M J (2003) Vanilloid Receptors Take a TRP Beyond the Sensory Afferent. *Pain* 105 (1-2), 5-9.
- [19] Doerr A (2014). Single-particle Electron Cryomicroscopy. *Nature Methods*. 11(1), 30.
- [20] Liao M, Cao E, Julius D, Cheng Y (2013) Structure of the TRPV1 Ion Channel Determined by Electron Cryo-microscopy. *Nature* 504 (7478), 107-112.
- [21] Liao M, Cao E, Julius D, Cheng Y. (2013) TRPV1 Structures in Distinct Conformations Reveal Activation Mechanisms. *Nature* 504 (7478), 113-118.
- [22] Mammalian Transient Receptor Potential (TRP) Cation Channels, Volume 2 editado por B. Nilius, Veit Flockerzi, book
- [23] Jung J, et al (2002) Agonist Recognition Sites in the Cytosolic Tails of Vanilloid Receptor. *J Biol Chem* 277 (46), 44448-54.
- [24] Caterina M J, Schumacher M A, Tominaga M, Rosen T A, Levine J D, Julius D (1997) The Capsaicin Receptor: a Heat-activated ion Channel in the Pain Pathway. *Nature* 389 (6653), 816-824.
- [25] Lee Y M, Kang S M, Chung J H (2012) The Role of TRPV1 Channel in Aged Human Skin. *J Dermatol Sci* 65 (2), 81-85.
- [26] Sanz-Salvador L, Andrés-Borderia A, Ferrer-Montiel A, Planells-Cases R (2012) Agonist- and Ca²⁺-dependent Desensitization of TRPV1 Channel Targets the Receptor to Lysosomes for Degradation. *J Biol Chem* 287 (23), 19462–19471.
- [27] Yi-Ching L, et al (2005) Capsaicin-induced Cell Death in a Human Gastric Adenocarcinoma Cell Line. *World J Gastroenterol* 11 (40), 6254-6257.
- [28] Lin C H, Lu W C, Wang C W, Chan Y C, Chen M K (2013) Capsaicin Induces Cell Cycle Arrest and Apoptosis in Human KB Cancer Cells. *BMC Complement Altern Med* 13 (46), 1-9.
- [29] Berridge M J, Lipp P, Bootman M D. (2000) The Versatility and Universality of Calcium Signalling. *Nat Rev Mol Cell Biol* 2000; 1: 11–21;
- [30] Berridge M J, Bootman M D, Lipp P (1998) Calcium – A Life and Death Signal. *Nature* 395 (6703), 645-648.
- [31] Prevarskaya N, Skryma R, Shuba Y (2011) Calcium in Tumour Metastasis: New Roles for Known Actors. *Nat Rev Cancer* 11 (8), 609-618.
- [32] Phelps C B, Wang R R, Choo S S, Gaudet R (2010) Differential Regulation of TRPV1, TRPV3, and TRPV4 Sensitivity Through a Conserved Binding Site on the Ankyrin Repeat Domain. *J Biol Chem* 285:731–740
- [33] Monteith G R, McAndrew D, Faddy H M, Roberts-Thomson S J (2007) Calcium and Cancer: Targeting Ca²⁺ Transport. *Nat Rev Cancer* 7 (7), 519–530.
- [34] Bano D, et al. (2005) Cleavage of the Plasma Membrane Na⁺/Ca²⁺ Exchanger in Excitotoxicity. *Cell* 120 (2), 275-285.
- [35] Schwab B L, et al. (2002) Cleavage of Plasma Membrane Calcium Pumps by Caspases: a Link Between Apoptosis and Necrosis, *Cell Death Differ* 9 (8), 818–831.
- [36] Reilly C A, Taylor J L, Lanza D L, Carr B A, Crouch D J, Yost G S (2003) Capsaicinoids Cause Inflammation and Epithelial Cell Death Through Activation of Vanilloid Receptors. *Toxicol Sci* 73 (1), 170–181.
- [37] Amantini C, et al. (2007) Capsaicin-induced Apoptosis of Glioma Cells is Mediated by TRPV1 Vanilloid Receptor and Requires p38 MAPK Activation. *J Neurochem* 102 (3), 977-990.
- [38] Caprodossi S, et al. (2011) Capsaicin Promotes a More Aggressive Gene Expression Phenotype and Invasiveness in Null-TRPV1 Urothelial Cancer Cells. *Carcinogenesis* 32 (5), 686–694.
- [39] Gonzales C B, et al. (2014) Vanilloids Induce Oral Cancer Apoptosis Independent of TRPV1. *Oral Oncol.* 50 (5), 437–447.
- [40] Caprodossi S, et al. (2011) Capsaicin Promotes a More Aggressive Gene Expression Phenotype and Invasiveness in Null-TRPV1 Urothelial Cancer Cells. *Carcinogenesis* 32 (5), 686–694.
- [41] Sánchez M G, et al. (2005) Expression of the Transient Receptor Potential Vanilloid 1 (TRPV1) in LNCaP and PC-3 Prostate Cancer Cells and in Human Prostate Tissue. *Eur J Pharmacol* 515 (1-3), 20-27.

- [42] Sanz-Salvador L, Andrés-Borderia A, Ferrer-Montiel A, Planells-Cases R (2012) Agonist- and Ca²⁺-dependent Desensitization of TRPV1 Channel Targets the Receptor to Lysosomes for Degradation. *J Biol Chem* 287 (23), 19462-19471.
- [43] Gawecka E, Viken O (2012) Postherpetic Neuralgia: New Hopes in Prevention with Adult Vaccination and in Treatment with a Concentrated Capsaicin Patch. *Scandinavian J Pain* 3 (4), 220-228.
- [44] Webster L R, Malan T P, Tuchman M M, Mollen M D, Tobias J K, Vanhove G F (2010) A Multicenter, Randomized, Double-Blind, Controlled Dose Finding Study of NGX-4010, a High-Concentration Capsaicin Patch, for the Treatment of Postherpetic Neuralgia. *J Pain* 11 (10), 972-982.
- [45] Singh R, Kishore L, Kaur N (2014) Diabetic Peripheral Neuropathy: Current Perspective and Future Directions. *Pharmacol Res* 80, 21-35.
- [46] Kalff K M, et al. (2010) Pre-treatment with Capsaicin in a Rat Osteoarthritis Model Reduces the Symptoms of Pain and Bone Damage Induced by Monosodium iodoacetate. *Eur J Pharmacol* 641 (2-3), 108-113.
- [47] Peters A A, et al. (2012) Calcium Channel TRPV6 as a Potential Therapeutic Target in Estrogen Receptor-negative Breast Cancer. *Mol Cancer Ther*, 11 (10), 2158-2168.
- [48] Sorlie T, et al. (2003) Repeated Observation of Breast Tumor Subtypes in Independent Gene Expression Data Sets *Proc Natl Acad Sci USA* 100 (14), 8418-8423.
- [49] Domotor A, et al (2005) Immunohistochemical Distribution of Vanilloid Receptor, Calcitonin-gene Related Peptide and Substance P in Gastrointestinal Mucosa of Patients with Different Gastrointestinal Disorders. *Inflammopharmacology* 13 (1-3), 161-177.
- [50] Hartel M, et al. (2006) Vanilloids in Pancreatic Cancer: Potential for Chemotherapy and Pain Management. *Gut* 55 (4), 519-28.
- [51] Czifra G, et al. (2009) Increased Expressions of Cannabinoid Receptor-1 and Transient Receptor Potential Vanilloid-1 in Human Prostate Carcinoma. *J Cancer Res Clin Oncol*, 135 (4), 507-514.
- [52] Yang S L, Cao Q, Zhou K C, Feng Y J, Wang Y Z (2009) Transient Receptor Potential Channel C3 Contributes to the Progression of Human Ovarian Cancer. *Oncogene*, 28 (10), 1320-1328.
- [53] Yang S, Zhang J J, Huang X Y (2009) Orai1 and STIM1 are Critical for Breast Tumor Cell Migration and Metastasis. *Cancer Cell*, 15 (2), 124-134.
- [54] Sherkheli M A, Vogt-Eisele A K, Bura D, Beltran M L R, Gisselmann G, Hatt H (2010) Characterization of Selective TRPM8 Ligands and their Structure Activity Response (S.A.R) Relationship. *J Pharm Pharm Sci* 13 (2), 242-253.
- [55] Zhang L, Barritt G J (2004) Evidence that TRPM8 is an Androgen-dependent Ca²⁺ Channel Required for the Survival of Prostate Cancer Cells. *Cancer Res* 64 (22), 8365-8373.
- [56] Wu T T, Peters A A, Tan P T, Roberts-Thomson S J, Monteith G R (2014) Consequences of Activating the Calcium-permeable Ion Channel TRPV1 in Breast Cancer Cells with Regulated TRPV1 Expression. *Cell Calcium* 56 (2), 59-67.
- [57] Reilly C A, Taylor J L, Lanza D L, Carr B A, Crouch D J, Yost G S (2003) Capsaicinoids Cause Inflammation and Epithelial Cell Death through Activation of Vanilloid Receptors. *Toxicol Sci* 73 (1), 170-181.
- [58] Schwab B L, et al. (2002) Cleavage of Plasma Membrane Calcium Pumps by Caspases: a Link Between Apoptosis and Necrosis. *Cell Death Differ* 9 (8), 818-831.
- [59] Capiod T (2011) Cell Proliferation, Calcium Influx and Calcium Channels. *Biochimie* 93 (12), 2075-2079.
- [60] Wu T T L, Peters A A, Tan P T, Roberts-Thomson S J, Monteith G R (2014) Consequences of Activating the Calcium-permeable Ion Channel TRPV1 in Breast Cancer Cells with Regulated TRPV1 Expression. *Cell Calcium* 56 (2), 59-67.
- [61] Sheng M, Dougan S T, McFadden G, Greenberg M E (1988) Calcium and Growth Factor Pathways of c-fos Transcriptional Activation Require Distinct Upstream Regulatory Sequences. *Mol Cell Biol* 8 (7), 2787-2796.
- [62] Chang W C, Nelson C, Parekh A B (2006) Ca²⁺ Influx through CRAC Channels Activates Cytosolic Phospholipase A2, Leukotriene C4 Secretion, and Expression of c-fos through ERK-Dependent and -Independent Pathways in Mast Cells. *FASEB J* 20 (13), 2381-2383.
- [63] Hess J, Angel P, Schorpp-Kistner M (2004) AP-1 Subunits: Quarrel and Harmony Among Siblings. *J Cell Sci.*, 117 (Pt 25), 5965-5973.
- [64] Shaulian E, Karin M (2002) AP-1 as a Regulator of Cell Life and Death. *Nat Cell Biol* 4 (5), E131-136.
- [65] Preston G A, Srinivasan D, Barrett J C (2000) Apoptotic Response to Growth Factor Deprivation Involves Cooperative Interactions Between c-Fos and p300. *Cell Death Differ* 7 (2), 215-226.
- [66] Marti A, et al. (1994) Protein Kinase A and AP-1 (c-Fos/JunD) are Induced During Apoptosis of Mouse Mammary Epithelial cells *Oncogene*. 9 (4), 1213-1223.
- [67] Madeo A, et al. (2010) c-Jun Activation is Required for 4-hydroxytamoxifen-induced Cell Death in Breast Cancer cells *Oncogene* 29 (7), 978-991.
- [68] Parkash J, Asotra K (2010) Calcium Wave Signaling in Cancer cells. *Life Sci.*, 87 (19-22), 587-595.
- [69] Stewart T A, Yapa K T, Monteith G R (2014) Altered Calcium Signaling in Cancer Cells. *Biochim Biophys Acta. pii: S0005-2736 (14), 00298-3*.
- [70] Roderick H L, Cook S J (2008) Ca²⁺ Signalling Checkpoints in Cancer: Remodelling Ca²⁺ for Cancer Cell Proliferation and Survival. *Nat Rev Cancer* 8 (5), 361-375.
- [71] Schreck R, Baeuerle P A (1991) A Role for Oxygen Radicals as Second Messengers. *Trends Cell Biol* 1 (2-3), 39-42.
- [72] Ermak G, Davies K J A (2002) Calcium and Oxidative Stress: from Cell Signaling to Cell Death. *Mol Immunol* 38 (10), 713-721.
- [73] Barrera G (2012) Oxidative Stress and Lipid Peroxidation Products in Cancer Progression and Therapy. *ISRN Oncol* 2012, 1-21.

- [74] Kang D, Hamasaki N (2003) Mitochondrial Oxidative Stress and Mitochondrial DNA. *Clin Chem Lab Med* 41 (10), 1281-1288.
- [75] Behrend L, Henderson G, Zwacka R M (2003) Reactive oxygen species in oncogenic transformation. *Biochem Soc Trans* 31 (Pt 6), 1441-1444.
- [76] Pani G, Galeotti T, Chiarugi P (2010) Metastasis: Cancer Cell's Escape from Oxidative Stress. *Cancer Metastasis Rev.* 29 (2), 351-378.
- [77] Klaunig J E, Kamendulis L M, Hocevar B A (2010). Oxidative Stress and Oxidative Damage in Carcinogenesis. *Toxicol Pathol* 38 (1), 96-109.
- [78] Konstantinov A A, Peskin A V, Popova E Y, Khomutov G B, Ruuge E K. (1987) Superoxide Generation by the Respiratory Chain of Tumor Mitochondria *Biochim Biophys Acta* 894 (1), 1-10.
- [79] Zhou Y, Hileman E O, Plunkett W, Keating M J, Huang P (2003) Free Radical Stress in Chronic Lymphocytic Leukemia Cells and its Role in Cellular Sensitivity to ROS-generating Anticancer Agents. *Blood* 101 (10), 4098-4104.
- [80] Wu T, Harder B G, Wong P K, Lang J E, Zhang D D (2014) Oxidative Stress, Mammospheres and Nrf2-new Implication for Breast Cancer Therapy? *Mol Carcinog.*
- [81] Verma S, Kesh K, Ganguly N, Jana S, Swarnakar S (2014) Matrix Metalloproteinases and Gastrointestinal Cancers: Impacts of Dietary Antioxidants. *World J Biol Chem* 5 (3), 355-376.
- [82] Wu C C, et al. (2006) Capsaicin Induced Cell Cycle Arrest and Apoptosis in Human Esophagus Epidermoid Carcinoma CE 81T/VGH Cells through the Elevation of Intracellular Reactive Oxygen Species and Ca²⁺ Productions and Caspase-3 Activation. *Mutat Res* 601 (1-2), 71-82.
- [83] Wiseman H, Halliwell B (1996) Damage to DNA by Reactive Oxygen and Nitrogen Species: Role in Inflammatory Disease and Progression to Cancer. *Biochem. J* 313 (Pt. 1), 17-29.
- [84] Cerutti P A (1994) Oxy-radicals and Cancer. *Lancet*, 344 (8926), 862-863.
- [85] Yang K M, et al. (2009) Capsaicin Induces Apoptosis by Generating Reactive Oxygen Species and Disrupting Mitochondrial Transmembrane Potential in Human Colon Cancer Cell Lines. *Cell Mol Biol Lett* 14 (3), 497-510.
- [86] Lakhani S A, et al. (2006) Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. *Science.* 311 (5762), 847-51..
- [87] Chou C C, Wu Y C, Wang Y F, Chou M J, Kuo S J, Chen D R (2009). Capsaicin-induced Apoptosis in Human Breast Cancer MCF-7 Cells through Caspase-independent Pathway. *Oncol Rep* 21 (3), 665-671.
- [88] Tuoya B N, et al. (2006) Apoptosis Induction by Dohevanil, a DHA Substitutive Analog of Capsaicin, in MCF-7 Cells. *Life Sci* 78 (13), 1515-1519.
- [89] Hwang J T, et al. (2005) Genistein, EGCG, and Capsaicin Inhibit Adipocyte Differentiation Process Via Activating AMP Activated Protein Kinase. *Biochem Biophys Res Commun* 338 (2), 694-699.
- [90] Kim S, Moon A (2004) Capsaicin-induced Apoptosis of H-ras Transformed Human Breast Epithelial Cells is Rac-dependent via ROS Generation. *Arch Pharm Res* 27 (8), 845-849.
- [91] Surh Y J, Lee S S (1995) Capsaicin, a Double-Edged Sword: Toxicity, Metabolism, and Chemopreventive Potential. *Life Sci* 56 (22), 1845-1855.
- [92] Vinitketkumnuen U, Sasagawa C, Matsushima T (1991) Mutagenicity Study of Chili and its Pungent Principles, Capsaicin and Dihydrocapsaicin. *Mutat. Res.* 252 (1), 115.
- [93] Lee B M, Park K K (2003) Beneficial and Adverse Effects of Chemopreventive Agents. *Mutat Res* 523-524, 265-78.
- [94] Muralidhara, Narasimhamurthy K (1988) Non-mutagenicity of Capsaicin in Albino Mice. *Food Chem Toxicol* 26 (11-12), 955-958.
- [95] Reilly C A, et al. Metabolism of Capsaicin by Cytochrome P450 Produces Novel Dehydrogenated Metabolites and Decreases Cytotoxicity to Lung and Liver Cells. *Chem Res Toxicol* 16 (3), 336-349.