Review article

Apoptosis-inducing activity and tumor-specificity of antitumor agents against oral squamous cell carcinoma

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Summary This review searched previous works of apoptosis induction in human oral squamous cell carcinoma (OSCC) cells. Many chemotherapeutic drugs, natural products, metabolic inhibitors, hormone receptor ligands and gene manipulations induced the apoptosis directly or indirectly by reverting the anti-apoptotic pathways. However, these antitumor agents, sometimes induced incomplete apoptosis or other types of cell death characterized by lack of caspase activation and internucleosomal DNA fragmentation, depending on the target cells or the chemical structure of inducers. There were very few investigations that have compared the cytotoxicity of these antitumor substances against OSCC cells with that of normal oral cells. Furthermore, their apoptosis-inducing activity has not been correlated well with tumor-specificity (higher cytotoxicity against tumor cells versus normal cells). These accumulated evidences provide the cautionary note against the apoptosis-oriented research.

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KEYWORDS
Oral squamous cell carcinoma; Apoptosis; Autophagy; Structure

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1. Introduction

Squamous cell carcinoma (SCC) is a form of cancer of the carcinoma type that may occur in many different organs, including the skin, lips, mouth, esophagus, urinary bladder, prostate, lungs, vagina, and cervix. It is a malignant tumor of squamous epithelium, and invasive cancers are able to spread to other organs and cause metastasis. Most cases of head and neck cancer are due to SCC, and caused by tobacco and alcohol, and human papilloma virus. Oral cancer is one of the most disfiguring types of cancer, since the surgical removal of the tumor may result in facial distortion.

Oral squamous cell carcinoma (OSCC) is the fifth most common cancer worldwide, with the number of cases consistently increasing in developing countries. Despite focused efforts to improve therapy, 5-year survival rates for persons with advanced-stage OSCC remain discouragingly low. OSCC, like other types of cancer, is a genetic disease, resulting in the loss of differentiation, and possibly generated by the decline of apoptotic potential and immunity [1]. Aggressive OSCC with a high score of malignancy showed reduced expression of the tumor suppressor gene phosphatase and tensin (PTEN) homologue deleted on chromosome 10 [2], p53 positivity and low apoptotic index [3], and increased expression of anti-apoptotic proteins such as survivin [4] and Bcl-2 [5]. Down-regulation of heat shock protein 27 enhanced the transformation of oral epithelial dysplasia into OSCC, possibly by impairing the protective mechanism against mutagenesis induced by environmental factors [6]. Carcinogenesis of OSCC is related to the overexpression of prolyl isomerase Pin 1 [7], a stronghold for the therapy of Alzheimer’s disease. These data suggest that OSCC may be produced by an imbalance of the regulation between cell survival and apoptosis.

Early detection combined with strategies for local intervention, such as chemoprevention prior to SCC development, could dramatically improve clinical outcomes. Microarray analysis of small noncoding microRNAs (miRNAs) in the patient can be a useful prognostic markers [8].

Recently, the targeted elimination of OSCC cells by inducing apoptosis has emerged as a valued strategy to combat oral cancer. Studies utilizing a variety of chemical or biological interventions demonstrated promising results for induction of apoptosis in oral malignant cells [9]. There are at least three types of cell death: apoptosis, autophagy and necrosis [10,11]. The present review surveys recent studies of the type of cell death (either apoptosis or non-apoptosis) induced by various antitumor agents in OSCC and the relationship to their tumor-specificity.

2. Classification of three major types of cell death

2.1. Apoptosis

Apoptosis is characterized by morphological changes such as cell shrinkage, pyknosis, densely packed cytoplasm, tightly packed organelles, chromatin condensation, and loss of cell surface microvilli [12]. Markers of apoptosis include DNA fragmentation (assessed by TUNEL method on fixed cell slide preparations, proportion of sub-G1 cells on FACS analysis, ladder pattern on agarose gel electrophoresis), decline of mitochondrial membrane potential, cytochrome c release from mitochondria, activation of caspase-3 using specific synthetic substrate or poly(ADP-ribose)polymerase (PARP).

2.2. Autophagy

Autophagic cell death is characterized by the sequestration of cytoplasm and organelles in double or multilamellar vesicles and delivery to the cell’s own lysosomes for subse-
quent degradation [13]. The process of autophagy depends on both continuous protein synthesis and the continuous presence of ATP [14]. Markers of autophagy is formation of phagophore and autophagosome detected by electron microscopy, Atg8/LC3 western blotting and ubiquitin-like protein conjugation systems (increase in the amount of LC3-II, and Atg12–Atg5 conjugation) or fluorescent microscopy (increase in punctuate LC3 (or Atg18), TOR and Atg1 kinase activity) [11].

2.3. Necrosis

Necrosis is an uncontrolled and passive process that usually affects large fields of cells. Necrotic cell injury is mediated by two main mechanisms, interference with the energy supply of the cell and direct damage to cell membranes [10]. Necrosis is characterized by cell swelling, formation of cytoplasmic vacuoles, distended endoplasmic reticulum, formation of cytoplasmic blebs, condensed swollen or ruptured mitochondria, disaggregation and detachment of ribosomes, disrupted organelle membranes, swollen and ruptured lysomes and eventually disruption of the cell membrane [15].

3. Apoptosis inducers

3.1. Apoptosis inducers against OSCC (Table 1)

3.1.1. Chemotherapeutic drugs

Chemotherapeutic drugs are the most frequently used apoptosis inducers of OSCC. 5-FU, peplomycin, cisplatin (DDP) and γ-ray, NO generator (ONOO-, SIN-1 and SNP) induced apoptosis in OSCC cells (SCC-4) [16]. These treatments slightly increased the intracellular concentration of NO and O₂⁻ levels, and induced the nitration of cytochrome c and caspase-3. DDP induced the rapid decline of surviving gene expression [17] and augmentation of apoptosome molecules (cytochrome c, Apaf-1) and activation of caspase-9 and capase-3, but no change in NF-kB nor the expression of anti-apoptotic proteins (TRAF-1, TRAF-2, c-FLIP). This suggests that cisplatin exerts its apoptotic action by the mitochondria-mediated activation of caspasas but not by the activation of caspasas due to the inhibition of NF-kB activity that follows the suppression of anti-apoptotic proteins [18]. Low-dose of CDDP enhanced the Fas(CD95) expression and increased susceptibility to apoptosis in oral cancer cell line (UT-SCC-20A) [19].

Carboplatin (CBDCA) induced apoptosis in human well-differentiated tongue squamous carcinoma cell lines (SCC-9 and SCC-25). The carboplatin-induced apoptosis was obliterated by neutralizing anti-Fas (APO-1/CD95) and anti-Fas ligand (FasL). Anti-Fas antibody itself was inactive, but in the presence of carboplatin, markedly enhanced the apoptosis. There was large enhancement of FADD expression, but no alterations in Fas or FasL expression upon carboplatin treatment. Suppression of FADD expression using the specific antisense oligonucleotide resulted in a failure of carboplatin induction of cell death. These data suggest that carboplatin may switch nonfunctional Fas to functional Fas by up-regulation of FADD expression, resulting in activation of Fas-sensitive pathway leading to apoptosis [20]. Carboplatin induced apoptosis in OSCC (MIT7) probably through the cleavage of Bax-alpha and Bcl-x(L) [21].

Paclitaxel, an antimitotic chemotherapeutic agent, induced apoptosis in human OSCC cell line (NB-1) by down-regulation of Bcl-2 and up-regulation of Bax [22]. Docetaxel induced Bax expression at both protein and mRNA levels in not all OSCC cell lines and clinical cases, but the enhanced expression of Bax has been suggested to be contributed to the antitumor effects of chemotherapy [23].

Peplomycin and bleomycin induced apoptosis in OSCC (SSKCN) [24]. Pingyangmycin (bleomycin A) induced growth arrest at G2-M phase of the cell cycle and apoptosis in OSCC (KB cells) [25].

137Cs irradiation, 5-fluorouracil (5-FU) or CDDP enhanced the apoptosis of OSCC induced by interleukin-2-activated lymphocytes [26]. Low-dose (0.5-Gy) of radiation caused enhanced apoptosis in human OSCC cells (T-167, T-409) without induction of transmembrane protein P-glycoprotein (MDR1), while higher dose (2-Gy) of radiation increased expression of MDR1 by NF-κB, NF-Y. Low-dose fractionated radiation can be used as an adjuvant for chemotherapy [27].

Heat stress induced 100-fold increase in the production of heat shock proteins, and prevented the NF-κB activation (nuclear translocation), may ultimately promote apoptosis in OSCC Ca9-22 cell line [28]. Hyperthermia induced apoptosis in OSCC cells (wild-type p53), accompanied by decrease of the gene expression of IL-12. On the other hand, IL-12Rβ1 increased in the mutated p53 cells. p53 status is a useful candidate for a predictive indicator of the effectiveness in hyperthermic therapy [29].

Molecular inhibition of epidermal growth factor receptor (EGFR) signaling is a promising cancer treatment strategy. Treatment of OSCC cells with an anti-EGFR monoclonal antibody, Cetuximab (C225, Erbitux) and an EGFR tyrosine kinase inhibitor (TKI), AG1478, which target the extracellular and intracellular domains of the receptor, respectively, inhibited phosphorylation of EGFR and its downstream effector molecule Akt and amplified the induction of Fas-mediated apoptosis. The pro-apototic activity of EGFR inhibitors in OSCC cells depends on the extrinsic pathway of the caspase cascade accompanied by the down-regulation of cellular FLICE-inhibitory protein (c-FLIP) [30].

3.1.2. Natural products

Essential oil of a perennial small herbal plant induced apoptosis via MAPK activation, based on the inhibition of MAPK kinase inhibitor [31]. Soft coral extract induced apoptosis in OSCC (SCC4, SCC9, SCC25) [32]. Ethanol extract of freeze-dried black raspberries suppressed the proliferation of OSCC cell lines without affecting viability, inhibited the production of vascular endothelial growth factor (VEGF), and induced both apoptosis and terminal differentiation. This extract may be a promising candidate for use as a chemopreventive agent in persons with oral epithelial dysplasia [33].

Theaflavin-3,3'-digallate, a polyphenol in black tea, induced apoptosis in HSC-2 cell by its prooxidant action (elevation of reactive oxygen species (ROS) production) whereas normal human gingival fibroblast (GN) was more resistant [34]. Green tea polyphenol induced apoptosis in OSCC cell line accompanied by the gradual decline of mitochondrial function. The cells deleted of caspase-3 gene did not undergo apoptosis. This suggests that green tea polyphenol-induced apoptosis is a mitochondria-targeted, caspase-3-executed mechanism [35]. Green tea polyphenols (catechins) induced
apoptosis in OSCC cells, and p58/KIP2 is a determinant pro-survival factor for cell protection from green tea polyphenol-induced apoptosis [36]. Curcumin alone or in combination with tea polyphenols inhibited the oral carcinogenesis in hamsters, possibly by suppressing the cell proliferation, induction of apoptosis and angiogenesis [37].

Stilbenes (resveratrol, piceatannol, rhaponticin), stilbene trimers (sophorastilbene A, (−)-α-viniferin), a stilbene dimer ((+)-α-viniferin) and flavonoids (kaempferol, fisetin, quercetin, isoliquiritigenin, butein) induced activation of caspase-3, -8 and -9, and internucleosomal DNA fragmentation in human promyelocytic leukemia HL-60 and OSCC (HSC-2) cells [38].

Ovatodiolide, a diterpenoid from a Chinese herb, induced G2/M arrest and apoptosis in OSCC cell line Ca9-22 by N-acetyl-cysteine (NAC)-inhibitable ROS generation [39]. Shi-G2/M arrest and apoptosis in OSCC cell line Ca9-22 by 8 and -9, and internucleosomal DNA fragmentation in human tin, isoliquiritigenin, butein) induced activation of caspase-3, -((+)ecoxib and NS-398 strongly suppressed the proliferation of KB lide, meloxicam) was compared using OSCC (KB) cells. Celecoxib and NS-398 strongly suppressed the proliferation of KB cells, accompanied by release of endonuclease G porine), a PKC inhibitor, also induced apoptosis and G1 arrest in OSCC cell lines. HSC-3 (primary-type OSCC) were less sensitive than LMF4 cells (metastatic-type OSCC) [47].

Protein phosphatase inhibitors, okadaic acid (OA) and calyculin A (CA), induced apoptosis in three OSCC cell lines (SCC-25, SCCKN, SCCFT) [48]. Okadaic acid induced apoptosis in OSCC (SCC-25) cells by enhancing the Fas and Fasl expression [49].

Flavopiridol, a synthetic flavone, inhibited the growth (by reducing the expressions of cyclin A, cyclin B and cyclin D1, and cyclin-dependent kinases (CDK) activation kinase and CDC25C) and induced apoptosis (by activating the Bcl-x pathway) in OSCC cells [50].

Proteasome inhibitors, carbobenzoxy-leucyl-leucyl-leucyl-norvalinal, or lactacystin, induced apoptosis in OSCC cells, but this was inhibited by antisense p27Kip1 oligonucleotide. The accumulation of p27Kip1 may play an important role in the apoptosis induced by proteasome inhibitor [51].

### 3.1.4. Hormone receptor ligands

15-Deoxy-D_{12}, Δ^{14}-prostaglandin (PG) J_{2}, a proliferator-activated receptor γ (PPARγ) ligand, induced growth inhibition and apoptosis in OSCC cells, whereas rosiglitazone and cigli-tazone, thiazolidinedione family of PPARγ activators, did not exert a growth inhibitory effect. 15-PGJ_{2} but not other PPARγ activators, induced significant reduction of both phosphorylated and unphosphorylated Stat3 (signal transducer and activator of transcription). This suggests that 15-PG induces apoptosis in OSCC by PPARγ-independent pathways [52].

Cyclopentenone 15-Deoxy-Δ^{12}, Δ^{14}PGJ_{2} induced apoptosis in OSCC (B88) cell lines by down-regulation of the constitutive and IL-6-mediated JAK (Janus kinase) phosphorylation as well as Stat3 phosphorylation. The apoptosis-inducing activity of this compound required the α,β-unsaturated ketone structure within the cyclopentenone ring [52].

- All-trans retinoic acid (ATRA) and 1α,25(OH)_{2}vitamin D_{3} (calcitriol) suppressed the cell proliferation and induced apoptosis in OSCC (KB) cells, upregulated sensitivity of the chemotherapeutics drugs and down-regulated several angiogenesis factors and survivin [53]. Retinoic acid induced apoptosis in OSCC (Tca83) cells by enhancement of Fas gene transcription and translation, without change in FasL expression [54].

### 3.1.5. Gene manipulations

RNAi targeting urokinase-type plasminogen activator receptor (u-PAR) induced apoptosis as well as oral cancer invasion and metastasis [55]. Stable transfection of intracellular fragment of Notch induced G0—G1 cell cycle arrest and apoptosis in human tongue cancer cell line (Tca8113), accompanied by down-regulation of the Wnt-β-catenin, increase of p21 and p53 expression, and decrease in Skp2 (S-phase kinase-associated protein 2) and Bcl-2 (B-cell lymphocytic-leukaemia proto-oncogene 2) expression [56].

### 3.1.6. Others

Nitric oxide (NO) donor, nitroprusside (SNP), induced keratinocyte differentiation markers at lower concentrations, while it induced apoptosis at higher concentrations in human immortalized keratinocytes (IHOK) and primary oral cancer cells (HK4) [57]. NO generator (ONOO\(^\cdot\), SIN-1 and SNP) induced apoptosis in OSCC (OSC-4) cells [16].
Selenium (Se) compounds induced apoptosis in OSCC (HSC-3) cells, accompanied by the loss of mitochondrial membrane potential and glutathione (GSH), without a concurrent increase in ROS. The apoptosis-inducing activity of Se was diminished by GSH or compounds that elevated the intracellular GSH level, but augmented by buthionine sulfoximine that reduced GSH level. These data suggest possible role of GSH in the mitochondrial apoptosis of OSCC caused by Se [58].

Arsenic trioxide induced toxic damage and apoptosis characterized by caspase-3 activation, mitochondrial transmembrane potential collapse and G2/M arrest, but no change in p16, p53 and Bcl-2, in OSCC cell line. Tubulins and mitochondria may be the chief action position of arsenic trioxide [59].

Taurolidine, a derivative of the amino acid taurine, induced apoptosis in two OSCC cell lines (SCC4, SCC15), in contrast to the reference group treated with povidone iodine or the untreated control group [60].

3.2. Head and neck squamous cell carcinoma (HNSCC) (Table 2)

Cetuximab is a chimeric mouse/human monoclonal antibody used to treat metastatic colorectal cancer and head and neck cancer. The antibody binds to the EGFR, a signalling protein that normally controls cell division. In some cancers, this receptor is altered to cause uncontrolled cell division. Cetuximab blocks EGFR and stops the uncontrolled cell division. Cetuximab induced apoptosis in HNSSC by inhibiting the MAPK and Janus kinase (JAK)/STAT-3 pathways [61].

Combination treatment with 13-retinoic acid, interferon-α2a and α-tocopherol apparently further inhibited the growth of five squamous cell carcinoma of the head and neck (SCCHN) in comparison to any single agent and two-drug combinations. Three-drug combination induced significant accumulation of the cells at S-phase and induced apoptosis. These data supported the promising outcomes of the phase III trial with a combination of these three drugs in patients with advanced head and neck cancer in the adjuvant settings [62].

Galanin is a neuropeptide present in humans and other mammals. It is a peptide consisting of a chain of 29 amino acids (or 30 amino acids in humans). Galanin is formed by the cleavage of a prepropeptide encoded by a gene known as GAL. Galanin induced apoptosis in galanin receptor-transfected HNSSC cell line with mutant p53 [63].

Alterations in histone acetylation status have been implicated in carcinogenesis. Histone deacetylase inhibitors, such as suberoylanilide hydroxamic acid, can potentially reactivate aberrantly silenced genes by restoring histone acetylation and allowing gene transcription. Suberoylanilide hydroxamic acid induced both mitochondrial pathway of apoptosis (cytochrome c release, caspase-3, caspase-9 activation) and extrinsic apoptosis pathway (increased Fas and Fas ligand (FasL) expression, activation of caspase-8, cleavage of Bid), having comparatively little activity against precancerous and normal oral cells [64].

Vitamin E succinate (α-TOS) induced apoptosis in five HNSSC cell lines (JHU-011, JHU-013, JHU-019, JHU-022, JHU-029) accompanied by elevation of ceramide, sphingomyelinase activity and p53. On the other hand, the amounts of nuclear factor κB, Bcl-2, and Bcl-X(L) proteins were reduced. Intraperitoneal administration of vitamin E succinate slowed the growth of JHU-022 solid tumor xenograft in immunodeficient mice [65].

An active component of OK-432, a streptococcal preparation, induced apoptosis in human HNSSC cell line by the activation of caspases through p53-independent pathway via TLR4 signaling [66].

The expression of E1A induced down-regulation of the expression of EGFR that was overexpressed in four HNSSC cell lines (Table 2). Overexpression of an exogenously introduced EGFR blocked E1A induction of apoptosis in these cells. These data suggest that E1A induces apoptosis by novel pathway involving EGFR [67].

Oral administration of vesnarinone, a cardiotonic, to a patient with histopathological recurrent oral cancer with well-differentiated squamous cell carcinoma, resulted in complete remission of the tumour [68].

3.3. Salivary gland carcinoma

Stindac, a non-steroidal anti-inflammatory drug (NSAID), induced a significant decrease in cell proliferation and an increase in apoptosis in two salivary gland adenocarcinoma cell lines (HSY, HSG) [69].

4. Anti-apoptotic mechanism in OSCC (Table 3)

OSCC (Ho-1-N-1 SP) cells survived even under treatment with various agents (5-FU, carboplatin), possibly due to high level expression of ABC transporters (ABC1, ABCG2) and anti-apoptotic protein (CFLAR, BCL2, BCL2A1) [70].

Hyperthermia-resistant human squamous cell carcinoma (SAS) cell: higher expression of Bcl-2, Bcl-xL, NF-κB, COX-2, STAT3, IL-6, IKKα/β] [71]. The imbalance between expression of anti-apoptotic and pro-apoptotic Bcl-2 family genes may promote survival in the oral cell lines [72]. The frequency of bcl-2 expression was associated with tumor histologic grade and decrease in apoptotic index in tongue SCC [73].

(--)Epigallocatechin-3-gallate (EGCG), a major component of green tea, induced p57, a cyclin-dependent kinase and apoptosis inhibitor, in a dose- and time-dependent manner in normal keratinocytes, but not in OSCC (SCC25, OSC2) cells. The chemopreventive effect of green tea polyphenols may involve p57-mediated cell cycle regulation in normal epithelial cell [74].

Paired box gene 9 (Pax9) and c-myb are transcription factors that regulate the expression of the genes involved in mediating cell proliferation, resistance to apoptosis, and migration. Pax9 and c-myb expressions in KB cells seem to be essential for cell growth, and survival was enhanced by c-myb. Disruption of the function of c-myb and Pax9 induced GO arrest [75].

The mouse double minute 2 (MDM2) plays a pivotal role in radiotherapy by down regulating p53. A functional T-to-G polymorphism at nucleotide 309 in MDM promoter intron 1 (SNP309) influences transcription activity. A G-to-C SNP at p53 codon 72 results in an Arg/Pro polymorphism, which is associated with apoptosis induction potential and p53 mutation status. The MDM2 SNP309 G/G polymorphism was associated with poor overall survival in advanced OSCC and the overall survival and disease-free survival of irradiated patients. The
The combination of MDM2 SNP309 G/G and p53 codon 72 Arg/Arg polymorphism is associated with the worst overall survival and disease-free survival. Both MDM2 SNP309 and p53 codon 72 SNP could be useful factors for evaluating the outcome of advanced OSCC treated with adjuvant radiation [76].

p58/KIP2 is a determinant pro-survival factor for cell protection from green tea polyphenol-induced apoptosis [36]. The nu/nu mice inoculated with Mn-SOD antisense-transfected SCC cells showed prolonged survival time, compared with mice inoculated with control vector-transfected SCC cells after treatment with antitumor agents such as 5-FU, peplomycin, CDDP or γ-ray. SCC cells transfected with Mn-SOD antisense group showed higher incidence of apoptosis than that transfected with empty vector. Mn-SOD may act as a negative regulator of apoptosis [77]. OSCC cell lines with lower Mn-SOD activity were easily committed to apoptosis upon treatment with 5-FU, peplomycin or γ-rays, than those with higher Mn-SOD. This suggests that Mn-SOD negatively regulates the apoptosis by these inducers [78].

5. Potentiators of apoptosis (Table 4)

Apoptosis induction may be potentiated by reversing the anti-apoptotic mechanism.

5.1. Chemotherapeutic drugs

CDDP administered for 24 h before 5-FU treatment for 48 h induced apoptosis in human oral cancer cells (888) more efficiently than simultaneous administration of CDDP and 5-FU or the sequential treatment of 5-FU followed by CDDP [79].

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Substances that induce apoptosis in OSCC cells.</th>
</tr>
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<tbody>
<tr>
<td>Inducers</td>
<td>Possible mechanism</td>
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<tr>
<td>Antitumor agents</td>
<td></td>
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<tr>
<td>5-FU</td>
<td></td>
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<tr>
<td>Cisplatin (CDDP)</td>
<td>Survivin ↑, FADD ↑, no change in NF-κB CDKIs ↑</td>
</tr>
<tr>
<td>Carboplatin (CBDCA)</td>
<td>Cleavage of Bax-alpha and Bcl-x(L)</td>
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<tr>
<td>Paclitaxel</td>
<td></td>
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<tr>
<td>Docetaxel</td>
<td>BAX ↑</td>
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<tr>
<td>Peplomycin, bleomycin</td>
<td>G2-M block</td>
</tr>
<tr>
<td>Radiation, γ-ray, Cs</td>
<td></td>
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<tr>
<td>Heat shock, hyperthermia</td>
<td>IL-12 ↓</td>
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<tr>
<td>Anti-EGFR mAb, EGFR TKI</td>
<td>c-FLIP ↓</td>
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<tr>
<td>Natural products</td>
<td></td>
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<tr>
<td>Essential oil</td>
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<tr>
<td>Soft coral extracts</td>
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<td>Black raspberry extract</td>
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<tr>
<td>Theaflavin-3,3′-digallate</td>
<td></td>
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<tr>
<td>Green tea polyphenols</td>
<td>p57/KIP2 ↓</td>
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<tr>
<td>Curcumin</td>
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<tr>
<td>Stilbenes, flavonoids</td>
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<tr>
<td>Diterpenoid</td>
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<tr>
<td>Shikonin, a naphthoquinone pigment</td>
<td>NF-κB ↓</td>
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<tr>
<td>Paradol</td>
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<tr>
<td>Inhibitors</td>
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<td>Selective COX-2 inhibitor, NS398, celecoxib</td>
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<tr>
<td>PKC inhibitor safingol, 7-hydroxyystaurorosporine</td>
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<tr>
<td>Phosphatase inhibitors: Okadaic acid calyculin A</td>
<td>Fas receptor ↑, FasL ↑</td>
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<tr>
<td>CDK inhibitor, flavopiridol</td>
<td>CDKs ↓</td>
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<tr>
<td>Proteasome inhibitors</td>
<td>p21Kip1 accumulation</td>
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<tr>
<td>Ligands to hormone receptors</td>
<td></td>
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<tr>
<td>Cyclopentenone 15-deoxy-Δ12, Δ14- PGJ2</td>
<td>JAK signalling ↓, Stat3 ↓</td>
</tr>
<tr>
<td>All-trans retinoic acid</td>
<td></td>
</tr>
<tr>
<td>1α,25(OH)2vitamin D3</td>
<td>Survivin ↓, VEGF ↓</td>
</tr>
<tr>
<td>Gene manipulation</td>
<td></td>
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<tr>
<td>siRNA targeting u-PAR</td>
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<td>Notch overexpression</td>
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<tr>
<td>Others</td>
<td></td>
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<tr>
<td>NO generator, ONOO−, SIN-1 and SNP</td>
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<tr>
<td>Selenium compounds</td>
<td>GSH ↓</td>
</tr>
<tr>
<td>Arsenic trioxide</td>
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<tr>
<td>Taurine</td>
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</table>
Tamoxifen alone induced a transient G1 arrest by up-regulation of cyclin-dependent kinase inhibitors (CDKIs) such as p21/Waf-1, p27/Kip1 and p15/INK4a in HNSCC cell lines (HN5, HN6), and sensitized the cells to apoptosis induced by CDDP. Tamoxifen stimulated the secretion of TGF-β1, and anti-TGF-β1 blocking antibody prevented both the blockade of cellular proliferation and increased expression of CDKIs. This suggests the role of TGF-β1 for the tamoxifen stimulation of cisplatin-induced apoptosis [80].

Hyperthermia is used as one of the treatment modalities for various types of cancers, but the acquisition of thermo-tolerance in cancer, through the induction of heat shock proteins (Hsps), renders hyperthermia less effective. Pretreatment of HSC-2 cells with IFN-γ suppressed Hsp27 transcription and promoter activity, and enhanced the induction of cell death by hyperthermia and cisplatin treatment [81].

5.2. Inhibitors

Etodolac, a selective COX-2 inhibitor, enhanced the carboplatin (CBDCA)-induced apoptosis of OSCC (SCC-25) cell line through the suppression of FAP-1 (anti-apoptotic tyrosine phosphatase) expression, although etodolac alone did not induce the apoptosis [82].

Histone deacetylase inhibitor, suberoylanilide hydroxamic acid, enhanced CDDP-induced apoptosis in the OSCC cell line (HSC-3), by rapidly up-regulating the endoplasmic reticulum (ER) stress-associated events such as the sustained phosphorylation of eukaryotic translation initiation factor-2 (eIF2), activation of protein phosphatase 1 and Akt dephosphorylation [83]. Co-administration of low-dose cisplatin (4 μg/ml) and suberoylanilide hydroxamic acid (2 μM) synergistically induced cytotoxicity and apoptosis in OSCC (Tca8113, KB) cell lines [84].

5.3. Others

Resveratrol (trans-resveratrol) is a phytoalexin produced naturally by several plants when under attack by pathogens treatment of HSC-2 cells with IFN-γ suppressed Hsp27 transcription and promoter activity, and enhanced the induction of cell death by hyperthermia and cisplatin treatment [81].
such as bacteria or fungi, and found in the skin of red grapes and is a constituent of red wine. Resveratrol down-regulated the expression of Bcl-2 and MDR1, and enhanced the sensitivity of human epidermoid carcinoma KBv200 cells [85].

Pretreatment with glycerol enhanced the effectiveness of X-irradiation in Ca9-22 cells bearing a mutant p53. Glycerol restored the DNA-binding activity of mutant p53 for a p53-consensus sequence to levels similar to that of wild-type p53 [86].

Infection with wild-type p53-encoding adenovirus alone, or X-irradiation alone, significantly inhibited the growth of OSCC (HSC-4 and SAS) cells, but combined treatment was most effective, even in mutant p53-accumulated HSC-4 cells [87].

ATLA and interferon-γ synergistically stimulated the apoptosis in OSCC cell lines [88].

6. Induction of non-apoptosis

As compared with the studies of apoptosis induction in OSCC, those of non-apoptosis induction are limited. Morin (3,5,7,2′,4′-pentahydroxyflavone) induced G2/M arrest, without induction of apoptosis in human OSCC cells, via inhibition of AKT activation [89]. Intratumoral laser illumination of OSCC transplanted nude mice induced necrosis, but not apoptosis, in OSCC cells [90].

We have recently reported that α,β-unsaturated ketones such as 1-trichloroacetyl-3-bromo-2-methoxynuizulene and 1-trichloroacetyl-3-chloro-2-ethoxynuizulene induced autophagic cell death characterized by the vacuolization detected by transmission electron microscopy, and the granular distribution of acridine orange and translocation of LC3-GFP into the autophagosome of OSCC (HSC-4) cells. Although HL-60 cells are easily committed to apoptosis by many inducers, this cell line did not express apoptosis markers, such as internucleosomal DNA fragmentation and caspase activation, upon treatment with 4,4-dimethyl-2-cyclopenten-1-one, α-methylene-γ-butyrolactone, 5,6-dihydro-2H-pyran-2-one, 3,3,3-trifluoro-2-hydroxy-1-phenyl-1-propanone, codeinone (an oxidative product of codeine) or morphinone (an oxidative metabolite of morphine) [91]. We found that 3,3,3-trifluoro-2-hydroxy-1-phenyl-1-propanone induced autophagic cell death (characterized by acridine and LC3-GFP accumulation in the autophagosome) in human OSCC cell lines (HSC-2, HSC-4) [92]. Furthermore, morphinone induced the formation of autophagosome engulfing organelles in HL-60 cells. The addition of N-acetyl-L-cysteine (NAC) reduced the cytotoxic activity of codeinone by 30-folds, whereas other anti-oxidants (cysteine, ascorbate, catalase) and metals (FeCl3, CoCl2, CuCl2) were almost inactive. Similarly, the cytotoxic activity of 4,4-dimethyl-2-cyclopenten-1-one, α-methylene-γ-butyrolactone, 5,6-dihydro-2H-pyran-2-one was almost completely eliminated by NAC. This suggests that the cytotoxicity of α,β-unsaturated ketones is generated by the interaction between the β-position of the α,β-unsaturated carbonyl moiety and SH group of any targeted molecules (the so-called "non-sterically hindered Michael acceptor"). We also found that morphinone induced a significant reduction of mitochondrial size. It is not clear at present whether this resulted from the perturbation of mitochondrial morphogenesis or the fragmentation of mitochondria as recently reported in Bax/Bak double knockout mouse embryonic fibroblast [93]. It has been recently reported that autophagy selectively degrades mitochondria, blocking the vicious cycle between defective mitochondria and ROS. These data suggest the mitochondrial target of autophagy.

7. Tumor-specificity (Table 5)

There are very few reports available that have dealt with the tumor-specificity of natural and synthetic compounds. Thaflavin-3,3′-digallate showed a concentration and time-dependent inhibition, with the OSCC cells more sensitive than the normal GN46 fibroblasts [34]. Morin showed higher cytotoxicity against OSCC than normal oral mucosa cells (NOMC) (TS = 1.6) whereas quercetin, kaempferol, datsisetin and galangin showed little or no tumor-specificity (TS = 1.03, 1.06, 1.00 and 1.08, respectively) [89]. Chemopreventive effects of green tea polyphenols may involve p57-mediated cell cycle regulation in normal epithelial cells [74]. Histone deacetylase inhibits suberoylanilide hydroxamic acid induced growth inhibition and apoptosis induction in HNSCC cell lines, but had limited effects on premalignant and normal cells [64]. Based on these backgrounds, we have surveyed a total of 1000 compounds for their tumor-specificity. The tumor-specific cytotoxicity index (TS) was determined by the ratio of the mean 50% cytotoxic concentration (CC50) against normal human oral cells (gingival fibroblast (HGF), pulp cell (HPC), periodontal ligament fibroblast (HPLF)) to that against human OSCC cell lines (HSC-2, HSC-3, HSC-4, NA, Ca9-22), submandibular gland carcinoma (HSG) [38,94,95] (Table 5). Preparations of normal cells from the extracted teeth and periodontal tissues have been approved by the intramural ethic committee after obtaining the informed consent from the donors. The compounds analyzed were mostly isolated and identified by our groups.

7.1. Flavonoid-related polyphenols

Flavonoids, generally, showed weak tumor-specificity (TS = 1.2–4.0). Licochalcone B, a chalcone derivative without an isoprenoid group, showed the highest TS value of 31.7. Isoprenoid-substituted chalcone, prenylated isoflavone and genistein had higher cytotoxicity, but without high tumor-specificity, suggesting that the prenylation itself is not necessary for the tumor-specificity. Among the benzophenones, compounds with two isoprenoid groups had higher cytotoxicity than the monoprenylated compound, but with minor TS values. Anthraquinones exhibited relatively higher TS values. Among them, emodin and aloe-emodin, without glycosylation, were the most potent (TS = 8.9 and >18.6, respectively). Stilbenes (resveratrol, piceatannol, raphonticin), stilbene trimers (sophorastilbene A, (−)-ε-viniferin), a stilbene dimer ((+)-α-viniferin) (TS = 1.4–3.6) and flavonoids (kaempferol, fisetin, quercetin, isoliquiritigenin, butein) (TS = 1.4–4.7) gave lower TS values.

7.2. Coumarins

Coumarin itself and its 7-hydroxy-, 6-methoxy-7-hydroxy and 5,6-dimethoxy-derivatives were relatively non-toxic to all cell lines. Its 6,7-dihydroxy derivatives (esculetin) revealed a tumor cell line-specific cytotoxicity (TS > 5.1). Higher tumor-specificity of 4-methyl (TS > 8.3), 3,4-dimethyl...
<table>
<thead>
<tr>
<th>Compounds</th>
<th>TS (range) (no. of compounds)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flavonoids</strong></td>
<td></td>
</tr>
<tr>
<td>Flavones, flavonols, isoprenylated flavonoids</td>
<td>1.2 ± 0.6 (0.3–3.2) (n = 36)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>3.3 ± 4.0 (0.8–31.7) (n = 27)</td>
</tr>
<tr>
<td>Isoprenylated flavonoids</td>
<td>2.1 ± 0.4 (1.6–3.0) (n = 22)</td>
</tr>
<tr>
<td>2-Arylbenzofurans</td>
<td>1.2 ± 0.2 (1.0–1.5) (n = 6)</td>
</tr>
<tr>
<td>Benzophenones</td>
<td>1.7 ± 0.4 (1.2–2.3) (n = 5)</td>
</tr>
<tr>
<td>Xanthones</td>
<td>1.3 ± 0.4 (n = 9)</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>3.8 ± 4.9 (1.0–18.6) (n = 13)</td>
</tr>
<tr>
<td>Phenylbutanone glucoside</td>
<td>2.4 (1.5–3.3) (n = 2)</td>
</tr>
<tr>
<td>Stilbene glucoside</td>
<td>1.8 ± 0.8 (1.0–2.6) (n = 3)</td>
</tr>
<tr>
<td>Naphthalene glucosides</td>
<td>1.3 (1.1–1.4) (n = 2)</td>
</tr>
<tr>
<td>Stilbenes</td>
<td>3.0 ± 1.2 (1.4–4.7) (n = 6)</td>
</tr>
<tr>
<td><strong>Tannin-related compounds</strong></td>
<td></td>
</tr>
<tr>
<td>Procyanidins</td>
<td>4.8 ± 2.3 (1.0–7.4) (n = 6)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>1.1 ± 0.1 (1.0–1.2) (n = 4)</td>
</tr>
<tr>
<td>Monohydrolyzable tannins</td>
<td>1.5 ± 0.5 (1.0–2.5) (n = 7)</td>
</tr>
<tr>
<td>Oligomeric hydrolysable tannins</td>
<td>1.4 ± 0.2 (1.2–1.5) (n = 3)</td>
</tr>
<tr>
<td>Macro cyclic ellagitanins</td>
<td>4.4 ± 2.7 (2.3–8.2) (n = 4)</td>
</tr>
<tr>
<td><strong>Terpenes</strong></td>
<td></td>
</tr>
<tr>
<td>Triterpenes</td>
<td>1.5 ± 0.7 (0.7–2.8) (n = 8)</td>
</tr>
<tr>
<td>Triterpene glycosides</td>
<td>1.4 ± 0.5 (1.0–2.4) (n = 21)</td>
</tr>
<tr>
<td>Triterpenes, triterpene glycosides, chromones</td>
<td>1.0 ± 0.1 (0.8–1.3) (n = 20)</td>
</tr>
<tr>
<td>Cycloartane glycosides</td>
<td>1.1 ± 0.2 (0.9–1.4) (n = 7)</td>
</tr>
<tr>
<td>Furostaol glycosides</td>
<td>2.5 ± 4.1 (0.4–17.0) (n = 17)</td>
</tr>
<tr>
<td><strong>Ketones</strong></td>
<td></td>
</tr>
<tr>
<td>α,β-Unsaturated ketones</td>
<td>1.2 ± 0.3 (0.6–1.9) (n = 26)</td>
</tr>
<tr>
<td>Cyclone α,β-unsaturated ketones</td>
<td>&gt;229.0 (n = 1)</td>
</tr>
<tr>
<td>α-Hydroxyketones</td>
<td>5.7 ± 6.0 (1.0–17.6) (n = 8)</td>
</tr>
<tr>
<td>β-Diketones</td>
<td>1.8 ± 1.4 (0.3–6.3) (n = 22)</td>
</tr>
<tr>
<td>Trifluoromethylketones</td>
<td>2.6 ± 1.6 (n = 6)</td>
</tr>
<tr>
<td>Azulenequinones</td>
<td>2.6 ± 2.3 (1.0–10.2) (n = 27)</td>
</tr>
<tr>
<td><strong>Bacterial products</strong></td>
<td></td>
</tr>
<tr>
<td>Anthracyclines</td>
<td>&gt;167 ± 89 (n = 4)</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>&gt;29 (n = 1)</td>
</tr>
<tr>
<td>Bleomycin, peplomycin</td>
<td>&gt;3.8 ± 0.2 (n = 2)</td>
</tr>
<tr>
<td>Nocobactines</td>
<td>62.0 (43.9–80.0) (n = 2)</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
</tr>
<tr>
<td>Vitamin K2 derivatives</td>
<td>1.9 ± 0.2 (1.7-2.0) (n = 3)</td>
</tr>
<tr>
<td>Prenylalcohols</td>
<td>1.3 ± 0.3 (1.0–1.8) (n = 5)</td>
</tr>
<tr>
<td>Azulenes</td>
<td>1.7 ± 1.0 (0.8–5.7) (n = 27)</td>
</tr>
<tr>
<td>Trihaloacetylazulenes</td>
<td>6.5 ± 10.7 (n = 26)</td>
</tr>
<tr>
<td>Tropolones</td>
<td>2.6 ± 1.8 (1.0–9.9) (n = 27)</td>
</tr>
<tr>
<td>Berberines</td>
<td>3.8 (3.6–4.0) (n = 2)</td>
</tr>
<tr>
<td>3,5-Dibenzoyl-1,4-dihydropyridines</td>
<td>&gt;43.0 (&gt;33 to &gt;53) (n = 2)</td>
</tr>
<tr>
<td>Styrylchromones</td>
<td>7.3 ± 6.1 (1.1–17.4) (n = 6)</td>
</tr>
<tr>
<td>Isoxazole derivatives</td>
<td>1.2 ± 0.2 (0.9–1.6) (n = 24)</td>
</tr>
<tr>
<td><strong>Antioxidants</strong></td>
<td></td>
</tr>
<tr>
<td>Epigallocatechin gallate (EGCG)</td>
<td>4.1 (n = 1)</td>
</tr>
<tr>
<td>Catechin</td>
<td>1.0 (n = 1)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>3.3 (n = 1)</td>
</tr>
<tr>
<td>Isoliquiritigenin</td>
<td>4 (n = 1)</td>
</tr>
<tr>
<td>Ascorbic acid (vitamin C)</td>
<td>2.5 (n = 1)</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>1.1 (n = 1)</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>1.7 (n = 1)</td>
</tr>
</tbody>
</table>
7.3. Tannins-related compounds

Procyanidin B-2 (MW 578) (TS = 5.8), procyanidin C-1 (MW 866) (TS = 6.7) and procyanidin oligomers (MW 3170) (TS > 7.4) had higher cytotoxicity and tumor-specific cytotoxicity against OSCC cells than did catechin (MW 290) (TS = 1.0), (-) -epicatechin (MW 290) (TS = 4.0) and (-) -epigallocatechin gallate (EGCG) (MW 458) (TS = 4.1).

Among hydrolyzable tannins (which contain glucose in the core of the molecule), three oligomeric hydrolyzable tannins (MW 1854−1873) had an approximately two-fold higher cytotoxicity than seven monomeric hydrolyzable tannins. Macro cyclic hydrolyzable tannins (oenothin B, woodfordin C, camellin B, woodfordin D) [38,94,95] (MW 1568−2506) exhibited one order higher cytotoxicity than monomeric hydrolyzable tannins. The tumor-specific cytotoxicity of macroyclic hydrolyzable tannins (TS = 2.7−8.2) was two- or three-fold higher than monomeric (TS = 1.0−2.5) or oligomeric hydrolyzable (TS = 1.2−1.5) tannins. On the other hand, gallic acid (MW 170) (TS = 1.1), methyl gallate (MW 184) (TS = 1.3), ellagic acid (MW 302) (TS = 1.0) and chlorogenic acid (MW 354) (TS = 1.7) had much lower tumor-specific cytotoxicity. These data suggest that a more condensed structure, with (hydrolyzable tannins) or without glucose in the molecule (condensed tannins such as procyanidin oligomers), increases the tumor-specificity.

(TS > 9.3) and 3,4-cycloalkyl derivatives (TS > 11.0) than 6,7-dihydroxycoumarin may be due to their higher lipophilicity.

7.4. Terpenoids

Generally, terpenoids had low tumor-specificity (TS = 0.5−2.4), except for oleanolic acid (TS > 2.8) and 22α-methoxyfurostanol monodesmosides (TS = 7.4, >17.4).

7.5. Ketones

The cytotoxic activity of α,β-unsaturated ketones declined with the introduction of methyl group at C-3 (β) or the addition of N-acetyl-L-cysteine (NAC), suggesting that their cytotoxicity is generated by the interaction between the C-3 and SH group of targeted molecules. Cideonine and morphine (oxidative metabolites of codeine or morphine, respectively) containing a α,β-unsaturated ketone backbone showed lower tumor-specificity (TS = 3.8 and 3.1, respectively). Cyclic α,β-unsaturated ketones, such as 3-aryliden-1-(4-nitrophenylmethylene)-3,4-dihydro-1H-naphthalen-2-ones exhibited unusually high tumor-specificity (TS > 229).

Among 8 hydroxyketones, deferiprone (TS > 17.6), mimosine (TS > 7.9), tropolone (TS > 4.1) and hinokitiol (TS = 10.7) had the highest tumor-specific cytotoxicity. On the other hand, maltol (TS = 1.0), kojic acid (TS = 1.4), 3-methyl-1,2-cyclopentanediene (TS = 1.3), 1,2-cyclohexanedione (TS = 1.2) had lower tumor-specificity.

Among 23 β-diketones, 3-formylchromone was the most tumor-specific (TS = 6.3), followed by (+)- and (−)-3-(tri fluorooacetyl)camphor (TS = 4.4), 4,4,4-trifluoro-1-phenyl-1,3-butadiene (TS = 3.4) and (−)-3-(trifluoroacetyl)camphor (TS = 3.3); others including curcumin were much less active (TS < 0.9−2.2).

Among 27 azulenequinone derivatives, 3-phenoxy-1,5 azulenequinone (TS > 8.5) and 7-isopropyl-3-(4-methylan lino)-2-methyl-1,5-azulenequinone (TS = 10.2) had highest tumor-specificity and apoptosis-inducing activity (caspase activation). Among 27 tropolone derivatives, 5-aminotropolone was the most tumor-specific (TS = 9.9) and had the highest apoptosis-inducing activity.

7.6. Synthetic compounds

Among 27 azulene derivatives, 2-acetylaminoazulene (TS > 3.6), diethyl 2-chloroazulene-1,3-dicarboxylate (TS > 5.7) and methyl 7-isopropyl-2-methoxyazulene-1-carboxylate (TS = 2.4) had the highest tumor-specificity. Chlorination of azulene resulted in the elevation of both cytotoxicity and tumor-specificity, while fluorination of the same compound was not so effective.

There was no apparent difference between the cytotoxic activity of 2-methoxyazulenes and 2-ethoxyazulenes. Trichloroacetylazulenes generally gave higher cytotoxicity and tumor-specificity as compared with the corresponding trifluoroacetylazulenes. Substitution of chloride, bromine or iodine at the C-3 position further enhanced their cytotoxicity to four tumor cell lines. Among 20 trihaloacetylazulene derivatives, 1-trichloroacetyl-3-bromo-2-methoxyazulene and 1-trichloroacetyl-3-chloro-2-ethoxyazulene had the highest tumor-specificity (TS = >3.5 and >2.5, respectively).

Berberines exhibited some tumor-specificity (TS = 3.6−4.0). Two 3,5-dibenzoyl-1,4-dihydropyridines had higher tumor-specificity, but only weakly induced apoptosis markers (DNA fragmentation, caspase activation). All six stryrylchromones had higher cytotoxic activity against tumor cell lines than against normal cells. Stryrylchromones, with one to three methoxy groups, had higher tumor-specificity and water solubility (TS = 5−17) and induced DNA fragmentation and caspase-3, -8 and -9 activation. Twenty-four 3-acetyl- and 3-benzylioxazole derivatives exhibited much lower tumor-specificity (TS = 0.9−1.6).

Table 5

<table>
<thead>
<tr>
<th>Compounds</th>
<th>TS (range) (no. of compounds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>1.7 (n = 1)</td>
</tr>
<tr>
<td>Morin</td>
<td>1.64 (n = 1)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1.06 (n = 1)</td>
</tr>
<tr>
<td>Kaemferol</td>
<td>1.06 (n = 1)</td>
</tr>
<tr>
<td>Datiscetin</td>
<td>1.00 (n = 1)</td>
</tr>
<tr>
<td>Galangin</td>
<td>1.08 (n = 1)</td>
</tr>
</tbody>
</table>

Data derived from [38,94,95].
Flavopiridol, a synthetic flavone, induced apoptosis (sub-G1, DNA content, DNA fragmentation, PARP cleavage) via activation of Bcl-x in OSCC cell lines [50].

7.7. Antitumor antibiotics

Doxorubicin, an anthracycline antibiotic isolated from Streptomyces peuceticus var. caesius has been used for the treatment of cancer of the bladder, breast (in combination with other anticancer agents) and prostate, but is, however, suspected to be a human carcinogen. Doxorubicin, used as a positive control in our screening system, exhibited the highest tumor-specific cytotoxic activity (TS = 255.0). It activated caspase-3, -8 and -9 in both OSCC (HSC-2) and promyelocytic leukemia HL-60 cells, but only induced internucleosomal DNA fragmentation in HL-60 cells. Western blot analysis showed that doxorubicin did not significantly change the intracellular concentration of Bcl-2, Bax or Bad in HL-60 cells. Real time PCR analysis showed that mdr1 expression in the tumor cells seems to be unrelated to the tumor-specificity of doxorubicin. Other anthracyclines such as mitoxantrone (TS > 259) and daunorubicin (TS > 164) produced comparable tumor-specificity with doxorubicin. Idarubicin exhibited slightly lower cytotoxicity and tumor-specificity (TS = 47). On the other hand, the tumor-specificity of other groups of antitumor antibiotics such as mitomycin C (TS > 29), bleomycin (TS > 25) and peplomycin (TS > 4.0) was much lower. This confirms the antitumor potential of anthracyclines for the treatment of OSCC.

Nocobactins NA-a (NBNAa) and NA-b (NBNAb) are mycobactin-like siderophores, which may play a role in the uptake of iron from the proteins of the host by chelation of ferric ion (Fe(III)). These compounds exhibited high tumor-specificity index (TS = 80.0 and 43.9, respectively).

7.8. Plant extracts

Poly-herbal extracts of Himalaya (HD-12, DLH-3073) exhibited highly tumor-specific cytotoxicity to tumor cell lines (TS > 1070 and >106, respectively) [96]. These extracts produced radicals under alkaline condition and scavenged O2·-. The tumor-specificity and antioxidant properties suggest their medicinal efficacy. The identification of the active principle is essential.

8. Conclusion

The present review article demonstrated that OSCC cells can be committed to either apoptosis or non-apoptosis, depending on the type or sensitivity of target cells. OSCC showed different susceptibility of apoptosis, upon treatment with 5-FU [97]. There was a considerable variation (approximately 10–20-fold) in drug-sensitivity of five OSCC cell lines. The sensitivity to mitomycin C is in the following order (from sensitive to resistant): HSC-2 (CC50 = 3.5 μM) > HSC-3 (9.7 μM) > Ca9-22 (16.4 μM) > HSC-4 (18.0 μM) > NA (37.8 μM). The sensitivity to bleomycin is in the following order: HSC-2 (CC50 = 4.6 μM) > HSC-3 (6.3 μM) > HSC-4 (77.4 μM) > NA (91.6 μM) > Ca9-22 (111.6 μM). The sensitivity to peplomycin is in the following order: HSC-2 (CC50 = 9.9 μM) > HSC-3 (25.2 μM) > NA (143.2 μM) > HSC-4 (175.8 μM) > Ca9-22 (216.9 μM) [94]. In general, OSCC cells are relatively resistant to Fas-mediated apoptosis; this may be due to a lower expression of FAS [5] or the cellular FLICE-inhibitory protein (c-FLIP) [98]. A cyclooxygenase (COX)-2 inhibitor (NS398) induced G0/G1 arrest, but no apoptosis in OSCC cells [99]. On the other hand, human glioblastoma cell lines (M059J, M059K, U373-MG and T98G) has been reported to more exclusively commit to autophagy (characterized by autophagosome formation, the accumulation of Agp8p/Aut7p and LC3 (Atg8 homolog) in autophagosome, and the inhibition of cell death by 3-methyladenine, an autophagic inhibitor), upon exposure to radiation (137Cs) [100], arsenic trioxide [101], ceramide [102] or temozolomide (a new alkylating agent) [103] or doxorubicin [104]. Anthracyclines, with the highest tumor-specificity, also induced non-apoptotic cell death in acute myeloblastic leukemia [105], cardiomyocytes [106] and breast cancer cells [107].

Another factor that determines the type of cell death is the category of compounds that is used. α,β-Unsaturated ketones (2-cyclohexen-1-one, 2-cyclopenten-1-one, 4,4-dimethyl-2-cyclopenten-1-one, 2-cyclohepten-1-one, α-methylene-γ-butyrolactone, 6-dihydo-2H-pyran-2-one, methyl 2-oxo-2H-pyran-3-carboxylate, codeinone, morphine) and 3,5-dibenzoyl-1,4-dihydropyridines activated caspase only marginally in HL-60 cells. Morphinone, an oxidative metabolite of morphine, similarly induced non-apoptotic cell death, accompanied by a significant reduction of mitochondrial size.

There is evidence that modulation of one form of cell death may lead to another. Various stimuli (such as etoposide, TNF, hyperthermia, UV irradiation, ascorbic acid, hydrogen peroxide) induced apoptosis (internucleosomal DNA fragmentation) in various human leukemia cell lines at relatively lower concentrations, whereas it induced necrotic cell death (smear pattern of DNA fragmentation) at higher concentrations [108]. Recently, cross-talk between apoptotic and autophagic pathways has been suggested [109,110].

This review reveals that tumor-specificity and apoptosis induction do not always correlate with each other. This means that having an apoptosis-inducing activity does not guarantee its antitumor activity. Similar is true for autophagy-inducing compounds, since α,β-ununsaturated ketones which exclusively induce non-apoptosis, have shown broad range of tumor-specificity (TS = 1–200). The tumor-specific cytotoxicity may be affected by various factors such as the co-existence of both hydrophilic and hydrophobic groups in the same molecule, the presence of an isoprenyl group, a halogen and/or a polycyclic structure, a highly condensed structure, and lipophilicity. The expression of multi-drug resistant proteins and drug metabolizing enzymes may also play an important role. External factors that may affect the tumor-specificity include the type of serum, oxygen concentration, metallic ion presence/concentration and external pressure. The cytotoxic activity of curcumin and nocobactins was significantly inhibited by the addition of FeCl3 due to the chelate formation. Systematization of the relationship between various factors mentioned above and tumor-specificity may contribute to the quest for more active compounds.
Both the resistance of tumor cells to anticancer drugs and the dose-related toxicity remain the most important problems in the chemotherapy of clinical OSCC. Researchers have been seeking a combinative treatment regimen to improve the effect of chemotherapy, 13-Retinoic acid, interferon-α2a and α-tocopherol synergistically induced apoptosis in squamous cell carcinoma of the head and neck (SCCHN) may be a basis of the promising outcomes of the phase III trial with a combination of these three drugs in patients with advanced head and neck cancer in the adjuvant settings [62].

Conflict of interest

There is no financial and personal relationships with other people or organizations that could inappropriately influence this review article.

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References

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