Review

Skin Pharmacology and Physiology

Skin Pharmacol Physiol 2018;31:59–73
DOI: 10.1159/000479529

Received: January 24, 2017
Accepted: July 13, 2017
Published online: December 21, 2017

Neoplastic Multifocal Skin Lesions: Biology, Etiology, and Targeted Therapies for Nonmelanoma Skin Cancers

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Keywords
Nonmelanoma skin cancers · Basal cell carcinoma · Squamous cell carcinoma · Actinic keratosis · Novel oral targeted therapies · Clinical trials

Abstract
Neoplastic skin lesions are multifocal, diffuse skin infiltrations of particular relevance in the differential diagnosis of ulcerative, nodular, or crusting skin lesions. Nonmelanoma skin cancers (NMSCs), namely, basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and also actinic keratosis (AK), are the most common malignant tumors in humans. BCCs do not proliferate rapidly and most of the times do not metastasize, while SCCs are more infiltrative, metastatic, and destructive. AKs are precursor lesions of cutaneous SCCs. The classical therapy of NMSCs makes use of photodynamic therapy associated with chemotherapeutics. With improved understanding of the pathological mechanisms of tumor initiation, progression, and differentiation, a case is made towards the use of targeted chemotherapy with the intent to reduce the cytotoxicity of classical treatments. The present review aims to describe the current state of the art on the knowledge of NMSC, including its risks factors, oncogenes, and skin carcinogenesis, discussing the classical therapy against new therapeutic options.

Introduction

Nonmelanoma skin cancers (NMSCs) (or keratinocyte carcinoma) are the most common malignant tumors in humans. Although the NMSC incidence is difficult to determine, because reliable registration of these cancers has not been achieved, some specific studies indicate that 1 in every 3 cases of cancer diagnosed in humans is NMSC. NMSC represents around 80% of the total amount of cutaneous cancers, with an incidence rate 20 times that of melanoma [1]. Despite growing public awareness of the harmful effects of sun exposure and health costs, NMSC incidence has been increasing by 4% each year, and currently 2–3 million NMSCs are diagnosed worldwide each year [2, 3].

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NMSCs comprise various types of carcinomas, such as basal cell carcinomas (BCCs), squamous cell carcinomas (SCCs), baso-SCCs, actinic keratoses (AKs), infiltrative carcinoma, Merkel cell carcinomas, adnexal tumors, cutaneous lymphomas, angiosarcomas, dermatofibrosarcomas protuberans, Bowen disease, erythroplasias of Queyrat and bowenoid papulosis, and atypical fibroxanthomas for example. BCCs and SCCs constitute the vast majority and occur in a ratio of 4:1 [4]. Other pathologies represent a relatively small proportion of diagnoses compared to BCCs and SCCs [5]. BCCs do not proliferate rapidly and most of the times do not metastasize, but, if ignored, they are prone to destroy local underlying tissues [6]. BCCs can be classified into various subtypes based on the location, gender, age, and skin type, which influence treatment choices and treatment responses. BCC subtypes are superficial, nodular, micronodular, morpheaform, sclerosing, infiltrative, and basosquamous. The less common BCC subtypes exhibit a significantly more aggressive biological behavior, such as sclerosing or metaplastic BCC or rodent ulcer, which occur more rarely [7]. The metastatic potential is low, but exposure to ultraviolet (UV) radiation increases the risk of developing this type of tumor [8]. SCC, the second most common skin cancer, arises from malignant proliferation of epidermal keratinocytes. It is characterized by infiltrative and metastatic behavior as well as destructive growth [9]. Several histological subtypes of SCC have been described, including keratoacanthoma, verrucous carcinoma (a less common but more invasive SCC variant), SCC in situ, also known as Bowen disease, acantholytic SCC, spindle cell SCC, clear cell SCC, papillary SCC, and signet ring, pigmented, and desmoplastic SCC [10]. In contrast to BCCs, SCCs tend to grow more rapidly (weeks to months) and have an overall metastatic rate of 2–3% [11]. The primary cutaneous SCC represents the second most common cancer among Caucasians. Only in the USA, an estimated 700,000 new cases occur during 1 year. Worldwide, approximately 650,000 patients with head-and-neck SCC are diagnosed yearly, thus it is considered the sixth most common cancer. Survival of patients with this cancer has not significantly improved; the 5-year survival is 40–50%, with approximately 223,000 deaths per year. In the case of metastatic head-and-neck SCC, survival prognosis is bleak (<1 year); 80% of head-and-neck SCCs derive from the oral cavity and oropharynx. This type of cancer is characterized by a high rate of local tumor extension, frequent distant metastases, and cervical lymph node metastases. Sun and UV radiation exposure and immunosuppressive medications are frequent risk factors for the development of cutaneous SCC. The outcome of this carcinoma is poor, with a 1-year survival of 44–56% [12]. The mechanism of SCC development is complex and comprises many phases, starting from proliferation, over apoptosis to differentiation. Tumor development and progression are determined by the interplay between these intricate processes [13]. In SCC, the metastatic rates are estimated as high as 5% [14]. In oral SCC, for instance, cell adhesion molecules (specifically CD44, members of the tumor necrosis factor, cytokine family like FasL and TRAIL, some interleukins, ILs, such as IL-6, IL-8, IL-12, and IL-23, vascular endothelial growth factor, and epidermal growth factor receptor, EGFR) play an important role in metastases [15]. The dermoscopic pattern of invasive SCC has been shown to depend on the grade of histopathological differentiation, namely (i) well-differentiated SCC displays signs of keratinization as opaque, yellow scales, a central mass of keratin, structureless white areas, and yellow keratotic follicular plugs surrounded by a white rim (white circle) and (ii) poorly differentiated subtypes commonly lack signs of keratinization, displaying a predominant red color, which results from dense vascularity [16]. Elderly white people, with a phenotype of red hair, blue eyes, and fair skin, who had been chronically exposed to UV radiation for a long time, have major probability of having SCC. Even in the absence of UV radiation exposure, this tumor is occasionally associated with nonhealing wounds or with chronic lesions which result from chronic immunoinflammatory processes [9].

Frequently, SCC develops from early stages, the so-called AK, also known as solar keratosis, SCC in situ – solar keratotic type, or keratinocytic intraepidermal neoplasia [17]. AKs are very common in sun-exposed areas of the skin in elderly patients, appearing as reddish macules that are often covered by yellowish scales. While AK is historically considered to be a precancerous lesion, some authors now view them as premalignant skin lesions that may develop into SCCs [18]. At the histological level, these lesions are characterized by dysplasia and consist of keratinocytes manifesting atypically enlarged, irregular, and hyperchromatic nuclei. Cellular atypia in AK affects at least the basal epidermal layer but not the full thickness of the epidermis, and it is often associated with differentiation disturbances such as increased keratinization [11]. Atypical keratinocytes show enlarged nuclei with hyperchromasia, dyskeratosis, and mitoses in any layer of the epidermis.

Figure 1 summarizes the major pathogenic pathways involved in nonmelanoma skin cancers. PTCH1 shows tumor suppression activity encoding a protein receptor of

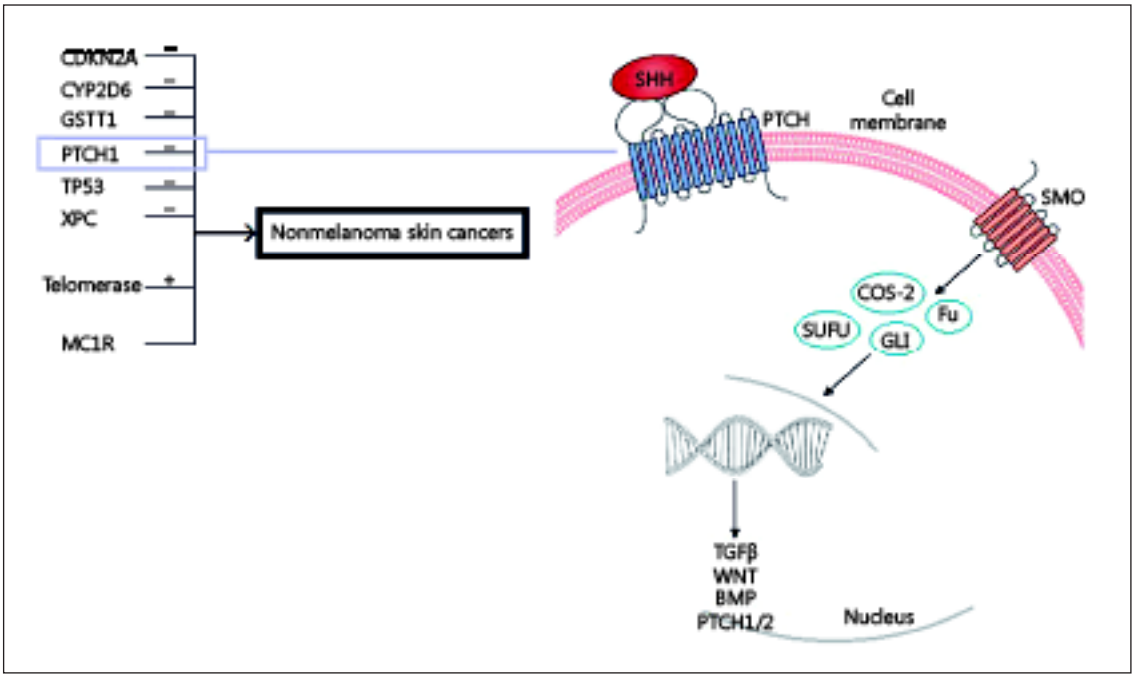

Fig. 1. Distinct pathways of pathogenesis of basal and squamous cell carcinoma. CDKN2A, cyclin-dependent kinase inhibitor 2A; CYP2D6, cytochrome P_{450}, family 2, subfamily D, polypeptide 6; GSTT1, glutathione S-transferase theta 1; MC1R, melanocortin 1 receptor; PTCH1, patched homolog 1; TP53, tumor protein 53; XPC, xeroderma pigmentosum, complementation group C. Human telomerase makes use of RNA to add telomere repeats at chromosome ends to compensate for telomere loss during cell division. Telomerase activity is high in most immortal and tumor cells, showing no net loss of telomere length during proliferation (modified from Madan et al. [19]).

the sonic hedgehog (SHH). The loss-of-function mutations of PTCH1 results in reduced suppression of intracellular signaling by the smoothened (SMO) G-protein-coupled receptor. SMO targets GLI transcription factors, while PTCH1 mutations induce sustained activation of target genes. A high frequency of somatic PTCH1 mutations is recognized in BCC, while up to 68% of BCC are sporadic mutations. Other relevant independent risk factors for NMSC are the MC1R (melanocortin-1 receptor) gene variants ASIP and TYR.

Risk Factors of NMSCs

NMSCs are known to encounter several risk factors (e.g., environmental, genetic, and phenotypic factors). NMSC are most frequent on parts of the body that are commonly exposed to the sun, such as ears, face, neck, and forearms, and affect mostly persons with fair skin and blue eyes, who burn rather than suntan when exposed to the sunlight. The long-term, repeated UV radiation exposure, both solar and artificial, is a causative factor for nearly 90% of NMSCs [2, 9, 20]. Exposure to UV radiation induces functional changes in keratinocytes and immune cells that lead to skin cancer [21]. Both UVB and UVA radiation have been shown to cause DNA damage and immunosuppression, the important forms of biological damage that lead to NMSCs [11]. UV light is thought to induce direct DNA mutation via covalent bonding between adjacent pyrimidines (UVB light) and formation of reactive oxygen species (UVA light) [22]. Within some countries (e.g., Australia), there is a clear relationship between increasing incidence of NMSCs with decreasing latitude, i.e., higher UV radiation levels [2, 11]. UV radiation causes direct DNA damage, but it can also result in the production of reactive oxygen species and reactive nitrogen intermediates which then cause indirect oxidative damage to the DNA [11]. Thus, sun avoidance and the use of sunscreen and sun-protective clothing are recommended to inhibit the initiation of this process [23]. Iatrogenic factors, such as radiation therapy or immunosuppressive therapy used in organ transplant patients, are

also known to increase the risk of NMSC [24]. Immunosuppressed patients are most likely to develop regional nodal metastases and to exhibit higher rates of tumors of adverse features and risk for death compared to the general population [25]. Specific immunosuppressive medications such as calcineurin inhibitors and azathioprine are also associated with a higher incidence of posttransplant SCCs [26]. These medications compromise immunity and lead to an increased susceptibility to infections and malignancies. After organ transplantation, the developed cutaneous malignancies are generally more aggressive and numerous than those seen in the general population [27]. Heart or lung transplants have been found to have a higher incidence of cutaneous SCC than liver transplants. However, liver transplants have been found to have a lower incidence of cutaneous SCCs when compared to other organ transplants [26].

Infection with human papilloma virus (HPV) of the β genus has also been implicated in the pathogenesis of posttransplant SCC [26]. However, a causal relationship between HPV and NMSC still needs to be established and is likely to be different from those found in other types of cancer, such as cervical cancers [28].

Genetic mutations are another important cofactor in the development of NMSC. The proliferation and differentiation of human keratinocytes is controlled by a large and coordinated range of genes. When sufficient mutations accumulate in many of these genes that significantly affect cell division, death, or differentiation, then this can be one step forward to NMSC [11]. Genes in which mutations lead to NMSC in this way are referred to as tumor suppressor genes and/or oncogenes [29]. Numerous tumor suppressor genes and oncogenes that are important for photocarcinogenesis have been identified. Examples include p53, PTCH1, RAS, BRM, GADD45, p16, c-Fos, Bcl-2, matrix metalloproteinases (MMPs), and mismatch repair genes [30, 31]. The most prominent and well-studied mutated gene highly associated with NMSC is the p53 tumor suppressor gene, found in 50–100% of all NMSC [32]. Polymorphisms of the melanocortin 1 receptor *(MC1R)* gene, in particular, correlate with fairness of skin, UV sensitivity, and enhanced cancer risk [20]. Other genetic factors known to increase a patient's risk of future skin cancer are inherited disorders such as xeroderma pigmentosum and nevoid BCC syndrome [33].

Important phenotypic features associated with an increased NMSC risk include age, male gender, skin and eye color (Fitzpatrick skin type I), history of severe sunburns, increasing number of melanocytic nevi and freckles, and a family history of skin cancer [2, 34, 35]. Naturally brown and black people can usually safely tolerate relatively high levels of sun exposure without getting sunburnt or greatly increasing their skin cancer risk. In contrast, people with pale or freckled skin, fair or red hair, and blue eyes belong to the highest risk group; people with dark hair and eyes who do not normally get sunburnt are at medium risk of developing skin cancer [36].

Oncogenes and Skin Carcinogenesis

Experimental mouse skin carcinogenesis has been widely used to identify the key molecular steps relevant in the carcinogenic process of tumor formation. In the mouse skin, chemically induced carcinogenesis is characterized by early appearance of a large number of benign papillomas. Then, these papillomas are followed by a much smaller number of invasively growing SCC.

A central dogma in cancer research is the concept of multistage carcinogenesis. The hallmark of the initiation of mouse skin carcinogenesis is the activation of mutations in Ras protooncogenes, where the majority of papillomas carry base substitutions in Ha-ras. These mutated alleles can be detected very early during carcinogenesis and are clonally expanded during tumor promotion [37].

In the cutaneous SCC, the sun-exposed skin and UV radiation can induce the generation of highly reactive oxygen species with the capacity to cause DNA damage and, thus, promoting mutagenesis. The cellular genomic integrity can be destabilized with an increased risk of acquiring additional cytogenetic alterations if the damage to DNA affects oncogenes, tumor suppressor genes, or cell cycle checkpoint control genes.

Uncontrolled cell proliferation, in response to microenvironmental growth signals, is facilitated by molecular alterations in oncogenes. The arrest of cell cycle required for DNA repair may be compromised by molecular changes in specific cell cycle stages. The effect may also be the avoidance of cell apoptosis as result of DNA damage. Given the limited capacity for DNA repair, transformed cells suffer division and propagation [9].

In the case of HPV infections, the oncoproteins E6 and E7 inhibit p53 and pRb tumor suppressor proteins, respectively. These proteins play a major role in the carcinogenesis of these viruses. Among immunosuppressed individuals, the β-papilloma viruses, such as HPV5, HPV8, and HPV9, are responsible for preneoplastic and neoplastic skin lesions. Currently, these viruses are detected in up to 90% of cutaneous SCCs observed in transplant recipients. Similar to different viruses, the HPV ge-

nome also depicts early genes encoding regulatory functions and late genes encoding structural components [26].

The molecular classification of melanomas is based on the most frequent driver oncogenic mutations (BRAF, NRAS, and KIT), and a long list of rare melanoma types has been established through genomic analyses. The mutation rate is significantly higher in melanomas than in other malignancies. Indeed, pediatric and hematological cancers have the lowest mutation rate (~1 mutations/Mb for chronic lymphocytic leukemia) [38] when compared to cancers with environmental mutagens, which are known to increase the mutation burden, such as melanoma and lung cancer (~15 mutations/Mb for melanoma) [39]. Moreover, continuous complex chromosomal alterations provide ample possibilities for adaptive genomics in various tissues and microenvironments. The phenotypic plasticity of melanoma is another characteristic that provides also a foundation for an extreme metastatic potential [40].

Classical Therapy of NMSCs

The goal of NMSC treatment is the complete eradication of the tumor while preserving important anatomical structures in terms of function and aesthetics [41]. Treatment options for NMSCs are based on patient and tumor characteristics, which determine whether a lesion shows low or high risk for cancer recurrence after treatment [10]. Chemosensitivity (measure of cell death by drug-induced apoptosis) and chemoresistance (quantity of tumor cell growth inhibition) assays would be of paramount importance to identify individual therapy options in these patients [42].

Surgery

Therapy of NMSCs may roughly be classified into surgical (physical: excisional surgery, laser ablation, cryosurgery, Mohs micrographic surgery [MMS], curettage with electrodessication, and radiation therapy) and nonsurgical (medical: medical therapy and photodynamic therapy).

Excisional surgery with predetermined margins is the mainstay of treatment for SCC and most BCC. Unlike many other destructive surgical techniques, surgical excision allows for histological examination of the excised tissue and accurate assessment of excisional margins. Apart from being highly effective (recurrence rate of <2% in 5 years after histologically complete excision of BCC), excisional surgery is usually associated with a good cosmetic outcome [43].

Lasers induce coagulative necrosis, ablation, and hyperthermia, leading to tumor destruction. Laser surgery presents advantages over conventional nonoptical ablation techniques, like a scalpel or electrosurgery, such as the increased precision of the resected volume, minimization of scars, and shorter recovery periods [44]. Many studies have provided evidence that lasers represent a new, effective treatment option for NMSC management [1, 45–47].

Cryosurgery induces localized frostbite, producing necrosis and tissue destruction, and it is indicated in patients with low-risk BCC lesions [48]. It is a quick and low-cost procedure; however, it may cause hypopigmentation or scarring [10].

MMS is a technique with proven efficacy and low local recurrence rates for many forms of skin cancer, including NMSC. The use of MMS has been steadily increasing during the past decade, with MMS representing approximately one-third of all surgical procedures for NMSC. MMS examines 100% of the surgical margin, which ensures definitive tumor removal in addition to sparing of uninvolved tissue, which promotes decreased recurrence rates, maximizing preservation of healthy tissue with more reconstructive options, and decreased overall costs [49–51].

Curettage and electrodessication is often used to treat small BCC at low-risk sites, i.e., the neck, trunk, and extremities. They are associated with high cure rates for BCC (95%) and SCC (92%) [52, 53]. Recurrence rates following this procedure are low at 3–6% for primary BCC <1 cm in size [54–56].

Radiation Therapy

Radiation therapy can be used successfully to treat NMSC and has the benefit of sparing normal, healthy tissue [57]. It is an alternative treatment for inoperable NMSC [58]. However, radiation therapy is not a treatment of choice for younger patients or for organ transplantation recipients who risk developing additional tumors in a treated field, and, therefore, it is usually reserved for patients who are poor surgical candidates [59].

Photodynamic therapy uses light activation of a tissue-localized photosensitizer in an oxygen-dependent process, which initiates the oxidative stress, inflammation, and cell death. The main advantage is the use of a source-narrow emission spectrum, which can correspond with maximum absorption photosensitizers [60]. Although approved for treatment of AK (a precancerous lesion of

Table 1. Oral drugs approved by the FDA for NMSC treatment

Generic name	Trade name	Dosage form (available dose)	Mechanism of action	Indication	Side effects
Vismodegib	Erivedge®	Capsule (150 mg)	Antagonist of the essential hedgehog pathway component Smoothened	Locally advanced (inoperable) and metastatic basal cell carcinoma (BCC)	Hair loss, weight loss, muscle cramps
Sonidegib	Odomzo®	Capsule (200 mg)	Smoothened inhibitor	Locally advanced BCC that has recurred following surgery or radiation therapy, or those who are not candidates for surgery or radiation therapy	Death or severe birth defects in a developing fetus when administered to a pregnant woman; serious musculoskeletal problems; increased serum creatine kinase levels; muscle pain and spasms

SCC), photodynamic therapy is associated with significantly higher BCC and SCC recurrence than standard therapies and is not approved by the Food and Drug Administration (FDA) for treatment of these tumors [61]. It is important to note that (unlike MMS and excisional surgery), curettage, electrodesiccation, radiation therapy, cryosurgery, and topical medications have one significant drawback in common. Since no tissue is examined under the microscope, there is no way to determine how completely the tumor was removed [62]. However, in selected cases, medical therapy, especially the recently developed molecular-targeted therapy, provides a high chance of cure and represents an alternative treatment approach to invasive procedures for some NMSC forms [6].

Oral Therapy

Oral therapy in NMSC is helpful in high-risk patients with recurrent and aggressive illness, who may not tolerate other systemic therapies [5]. Oral therapies which have shown at least partial efficacy include retinoids (isotretinoin, etretinate, acitretin, retinoic acid in combination with interferon-α, and cisplatin), 5-fluorouracil (5-FU), capecitabine, nonsteroidal anti-inflammatory drugs (NSAIDs), difluoromethylornithine, epigallocatechin gallate, sylimarin, curcumin, and lycopene. Additionally, the FDA has approved the use of 2 SMO inhibitors, vismodegib and sonidegib, in the therapy of BCC (Table 1).

The inhibition of the hedgehog pathway has been explored for local treatment of advanced and metastatic SCCs [63], giving rise to a new generation of pharmaceuticals, in particular vismodegib and sonedegib. Vismodegib is an inhibitor of the hedgehog pathway that blocks the effects of SMO homologues, which was the first to be approved, under a priority review program by the FDA and the European Medicines Agency. It is indicated for local treatment of advanced and metastatic BCC that has reappeared following surgery or which is incurable by surgery or radiation due to the considerable deformity or loss of function [63]. However, further studies are needed in order to discriminate the differences in tumor response between locally invasive and metastatic BCCs. This concern is extremely important if other hedgehog inhibitors show increased efficacy in metastatic disease or if other different pathways could be explored for metastatic disease targeting [5].

Sonidegib, also known as erismodegib, is another SMO homologue antagonist that was, recently, the second to receive FDA approval for cases of locally advanced BCC with recurrent disease, or for patients who do not qualify for radiation therapy or surgical removal [63]. It has demonstrated comparative results to vismodegib for locally advanced BCC [64]. It is suggested that commercial sonidegib capsules should be formulated by wet granulation in order to increase its absorption [5].

Retinoids are well known and widely tested as single agents in clinical trials for the chemoprevention and treatment of NMSC. They interfere with cell proliferation and differentiation through their interaction with specific cellular and nuclear receptors [65]. Systemic retinoids can be administrated to treat precancers and cancers, but more often they are used as chemoprevention due to reported side effects [66–71]. The most commonly administered systemic retinoids in chemoprevention of skin cancer are: etretinate, acitretin, and isotretinoin [72]. Depending on the dose, the use of retinoids may cause mild

(mucocutaneous reactions, hyperostotic axial skeletal changes, mild hair loss, skin xerosis, cheilitis, dry eyes, conjunctivitis, nasal dryness, epistaxis, and irritant dermatitis) to very serious side effects that are unpredictable, rare, dependent on individual susceptibility and predisposing factors (mucocutaneous toxic effects, elevated liver enzymes and liver dysfunction, increased levels of triglycerides and cholesterol, increased LDL fraction and decreased HDL fraction, risk of acute pancreatitis, and hepatotoxicity, skeletal abnormalities, demineralization, calcification of ligaments and vertebral disks, hyperostotic skeletal changes such as enlargement of existing bone spurs, and teratogenic effects) [69–71]. In that sense, it is advised to follow the patients closely in order to manage side effects, adjust the dosage, and monitor therapy effectiveness [68]. Moreover, the patients who undergo long-term retinoid therapy are advised to undergo periodic radiography to avoid side effects and toxicity [73]. An alternative method to control side effects of retinoids can be lowering the dose or the combination of the systemic retinoids with other compounds. For example, oral retinoic acid in combination with interferon-α and cisplatin was found to produce objective response rates in locally advanced and metastatic SCC [74].

5-FU is an antimetabolite that binds to thymidylate synthase, thereby inhibiting the conversion of deoxyuridine to thymidine nucleotides, which reduces DNA and RNA synthesis, decreases cell proliferation, and induces apoptosis [18]. Oral administration of 5-FU is not as common in treating advanced SCC, but if surgery fails and the additional side effects associated with intravenous chemotherapy are not ideal for a patient population, then oral administration is a viable option [5].

Capecitabine is a prodrug of the inactive form of 5-FU. The data presented in the previous case reports support the use of capecitabine for the chemoprevention of SCC in solid organ transplant recipients on prolonged immunosuppressive therapy [75–77].

NSAIDs and vitamin D derivatives specifically target hedgehog and/or wingless signaling pathways, which are activated in BCC. Diclofenac is a potent NSAID which acts by downregulating cyclooxygenase enzymes (primarily COX-2) and increasing apoptosis. Moreover, a relationship between the expression of COX-2, with its subsequent increase in prostaglandin levels, and the development of UVB-induced skin cancer has been shown. Fischer et al. [78] showed a decrease in prostaglandin synthesis in the epidermis, as well as a statistically significant decrease in tumor yield following the use of celecoxib (a specific COX-2 inhibitor).

Several studies have shown difluoromethylornithine as a chemopreventive agent against NMSC development [79–83]. It is a specific, irreversible inhibitor of ornithine decarboxylase, an enzyme that regulates tumor development [83]. Difluoromethylornithine has also been examined in combination with other potential chemopreventive agents (piroxicam and sulindac) [84, 85].

The grape seed polyphenolic antioxidant, sylimarin, curcumin, and lycopene, although not studied as extensively, appear to have also possible chemopreventive effects [73].

Topical Therapy

Topical therapy may represent an appropriate treatment method for therapy of NMSC, because it is not invasive, may reach a wide skin area, and does not cause hypertrophic scars. Topical treatments are preferable to invasive procedures, especially in the case of multifocal lesions, unclear lesion edges, risk of keloids, surgical risk factors, and localization in some areas such as the face and decolletage, as the cosmetic outcomes are generally excellent [6]. It represents an effective alternative treatment for superficial skin cancer, primarily AK and BCC [65]. Currently, topical agents, like 5-FU, imiquimod, ingenol mebutate, and diclofenac, have successfully demonstrated efficacy against AK and superficial BCC. These medications have FDA approval for the treatment of NMSCs (Table 2) that are confined to the epidermis.

Topical formulations of 0.5% (cream), 1% (cream), 2% (solution), and 5% (cream and solution) 5-FU are available in most countries for the treatment of skin cancer and are approved by the FDA for the treatment of multiple AK [6]. 5-FU has been shown to be particularly effective for treating thin (superficial) BCC when conventional methods are impractical (e.g., in the cases of multiple lesions or at difficult treatment sites) or when used in combination with curettage and electrodessication [6, 86].

Imiquimod is a synthetic imidazoquinoline amine that was the first of a new class of topical immune response modifiers [65]. Imiquimod is approved by the FDA to treat superficial BCC with a maximum diameter of 2 cm on the neck, trunk, or extremities (excluding hands or feet) and SCC in situ. Imiquimod is currently available in topical formulations of 2.5, 3.75, and 5% cream (Table 2). All formulations are approved by the FDA for face and scalp AK in immunocompetent individuals [10]. Topical 5-FU or imiquimod may be used before surgical excision of a tumor to reduce tumor size or for postsurgical removal of invasive tumors to treat remaining superficial tumor [10].

Table 2. Topical drugs approved by the FDA for NMSC treatment

Generic name	Trade name	Dosage form (drug concentration)	Mechanism of action	Indication	Common side effects
5-Fluorouracil	Carac® Efudex® Efudex® Fluoroplex® Tolak®	Cream (0.5%) Cream (5%) Solution (2%, 5%) Cream (1%) Cream (4%)	Blocks the methylation reaction of deoxyuridylic acid to thymidylic acid and creates thymine deficiency that provokes unbalanced growth and death of the cell	AK, sBCC	Cutaneous irritation (skin redness, inflammation, swelling, crusting)
Diclofenac	Solaraze®	Gel (3%)	Likely relates to its nonspecific inhibition of the cyclooxygenase isoenzymes COX-1 and COX-2	AK	Dry skin; flu-like symptoms; peeling, scaling, or flaking of the skin
Imiquimod	Aldara® Zyclara®	Cream (5%) Cream (2.5%, 3.75%)	Immune response modifier (stimulates the immune system to produce interferon, a chemical that destroys cancerous and precancerous cells)	AK, sBCC	Skin redness and ulcerations
Ingenol mebutate	Picato®	Gel (0.015%, 0.05%)	Induction of rapid cellular death followed by an inflammatory response	AK	Skin redness, flaking/scaling, crusting, swelling

AK, actinic keratosis; sBCC superficial basal cell carcinoma.

Ingenol mebutate, a macrocyclic diterpene ester, has a dual mechanism of action: the induction of rapid cellular death followed by an inflammatory response. Two formulations of ingenol mebutate are available and approved by the FDA for the treatment of AK: a 0.015% gel for face and scalp lesions and a 0.05% gel for the trunk and extremities. Moreover, experimental studies suggested a possible use of ingenol mebutate for the treatment of BCC [87, 88]. A promising chemopreventive agent for topical therapy is diclofenac. Its mechanism of action diverges from the available noninvasive therapies, which highlights its potential importance. A topical formulation containing diclofenac 3% gel in 2.5% hyaluronic acid is approved by the FDA for the treatment of AK [65]. A recent study evaluated the effectiveness of topical diclofenac sodium 3% gel, calcitriol 3 g/g ointment, and a combination of both in superficial and nodular BCC. Topical calcitriol was not effective; however, the topical administration of diclofenac during 8 weeks substantially reduced expression of proliferation and antiapoptosis immunohistochemical markers, and it also allowed for the complete tumor reversion of approximately two-thirds of cured superficial BCC. Other studies, using variable concentrations, excipients, or other combinations of considered drugs may be profitable in the optimization of new treatments. Thus, although surgical excision is still the best option for all BCC, topical gel diclofenac constitutes an auspicious noninvasive new approach for low-risk superficial BCC [89]. Like diclofenac, piroxicam has been shown to exert antitumorigenic effects. Campione et al. [90] investigated the use of piroxicam 1% gel in the treatment of AK. The application of piroxicam gel produced a complete response in 48% of the lesions and a partial response in 39% of the lesions. According to in vivo studies, piroxicam gel appears to be safe, effective, and well tolerated, whereas adverse effects did not require the treatment to be suspended [90].

There are a number of studies which evaluated the effectiveness of topical retinoids in the prevention and treatment of NMSCs, primarily AK [91–101]. The advantage of using topical retinoids is systemic toxicity avoidance, although there may be local adverse effects such as erythema, scaling, burning, and irritation which vary with the drug concentration. Topical retinoids used in the treatment of skin cancer include retinol, retinaldehyde, tretinoin (0.05 and 0.1% cream), isotretinoin (0.1% cream), and 2 third-generation retinoids, namely adapalene (0.1 and 0.3% gel) and tazarotene (0.1% gel) [65]. Despite numerous studies in the literature concerning retinoids in chemoprevention of NMSC, none of these

formulations have been approved by the FDA for the treatment of NMSCs, but they have been used off-label either alone or in combination with other modalities if the benefits appear greater than the risks [17, 102].

Resiquimod, an immune response modifier belonging to the same family as imiquimod, has been investigated for the topical treatment of facial and balding scalp AK. Resiquimod gel provided complete clearance varying from 40 to 74.2% after 1 course and from 77.1 to 90.3% after 2 courses [103].

T4 endonuclease 5 is an enzyme involved in the reparation of cyclobutane pyrimidine dimers generated in the DNA molecule after exposure to UV, which have been associated with NMSC generation [104]. T4 endonuclease 5 is being investigated as chemopreventive agent in humans with xeroderma pigmentosum having AK and BCC. Results showed that T4 endonuclease 5 encapsulated in a pH-sensitive engineered liposome and applied topically as lotion lowered the rate of new AK and BCC compared with the placebo lotion by >68 and 30%, respectively, during 1 year of treatment [104, 105].

Polyphenolic antioxidants, such as epigallocatechin gallate, has been investigated in numerous studies that involve skin carcinogenesis. Though their exact mechanisms of action are not precisely known, studies on the inhibitory effect of both topically and orally applied epigallocatechin gallate on tumor initiation and promotion have shown that they effectively protect against both chemical carcinogen- and UVB irradiation-induced skin tumorigenicity by means of the inhibition of a specific transcription factor, but they also decrease related conventional measurements of chemical carcinogenesis (i.e., enzyme activity, epidermal edema, and hyperplasia) [106–109].

Novel Therapeutic Options for NMSCs

Until a few years ago, the therapeutic approach for patients with advanced or metastatic NMSC was exclusively represented by conventional chemotherapy. The rising incidence of NMSCs has generated great interest that encouraged the development of new therapeutic agents. Recent studies on the pathogenesis of NMSC have suggested the use of molecular-targeted therapies as an alternative to conventional chemotherapy [6, 41].

Targeted Therapy

In the case of advanced and metastatic NMSC, molecular-targeted therapy represents a reasonably promising alternative to classical cytotoxic chemotherapy [6]. Targeted therapy stops the action of molecules that are key to the growth of cancer cells and affects cancer cells more than normal cells. Two main types of targeted therapy can be distinguished: the first type are small molecule drugs, which are small enough to enter cells, and the second type are monoclonal antibodies, which are too large to enter cells. Monoclonal antibodies affect targets outside of cells or targets on the cell surface.

The mechanism of action of monoclonal antibodies consists in the inhibition of the EGFR. EGFR is a tyrosine kinase receptor that, when activated, triggers several effects on keratinocytes, for example, cellular proliferation, enhancement of epidermal thickness, and keratinocyte resistance to programmed cell death [5]. Cetuximab attaches to the end of EGFR that is outside the cell to block EGF. Its efficacy has been proven in SCC treatment with a better tolerability in comparison to classical platinum- or fluoropyrimidine-based therapy [41]. Cetuximab has been approved by the FDA as injection for intravenous infusion (Erbitux®) for (i) the initial treatment of locally or regionally advanced SCC of the head and neck in combination with radiation therapy; (ii) the first-line treatment of patients with metastatic SCC in combination with platinum-based therapy in combination with 5-FU, and (iii) recurrent or metastatic SCC of the head and neck progressing after platinum-based therapy [110]. Gefitinib and erlotinib constitute first-generation EGFR tyrosine kinase inhibitors due to their robust response rates and progression-free survival in patients with adenocarcinomas. They have an anilinoquinazoline structure and have the capacity for binding reversely to adenosine triphosphate [111]. Erlotinib has found to be important in the case of failed previous implemented therapies [5]. New clinical trials are being conducted using erlotinib as a neoadjuvant and adjuvant therapy to surgery or radiation in aggressive cutaneous SCC [112]. Gefitinib has also been evaluated in the treatment of advanced SCC. It was shown that 45.5% of patients had an observable response to gefitinib, proposing its partial efficacy in targeting EGFR by using monotherapy. Response rates for gefitinib were highest in patients with only local disease (93%), and this, additionally, undermined the role for tyrosine kinase inhibitor monotherapy in metastatic or local disease [5]. Recently, statistical noninferiority in progression-free survival in patients with lung adenocarcinoma was found between erlotinib and gefitinib [111].

Dacomitinib, an orally bioavailable, covalent binding inhibitor of all enzymatically active HER family tyrosine kinases, has shown very recently favorable results over

erlotinib for EGFR activation mutations. Furthermore, Razak et al. [113] have found that the use of dacomitinib as first-line therapy in recurrent/metastatic SCC of the head and neck produced clinically meaningful disease control which is similar to the control achieved using cetuximab, but without the inconvenience of intravenous access and infusion-related reactions. Besides dacomitinib, afatinib – a novel, oral, irreversible tyrosine kinase inhibitor – has demonstrated benefits in patients with recurrent or metastatic SCC of the head and neck after failure of platinum-containing therapy and compares favorably to patients receiving cetuximab [114].

Combination Therapy

Combination therapy of bevacizumab (a vascular endothelial growth factor receptor inhibitor) with erlotinib (EGFR tyrosine kinase inhibitor) to erlotinib monotherapy provides another promising therapeutic option to treat patients with EGFR-activating mutations. There are no standard treatments for patients who acquire secondary resistance to EGFR tyrosine kinase inhibitor therapy, though promising new agents such as rociletinib are investigated in clinical trials. [115]. Tyrosine kinase inhibitors are expected to be approved by the FDA in order to be applied for the treatment of advanced SCCs which have previously failed standard treatments.

New targets have been elucidated for dual combination oral therapy targeting both EGFR and insulin-like growth factor 1 receptor, due to the lower response rates verified when using monotherapies. Picropodophyllin is an example of an insulin-like growth factor 1 receptor tyrosine phosphorylation inhibitor, which has shown efficiency in the inhibition of uveal melanoma growth after oral administration in mice [5].

Different discovered mutations and the overproduction of enzymes have been identified recently as possible targets for the inhibition of carcinogenesis, like NOTCH and FAM84B pathways and MMP-7 and IL-24 overproduction [5, 116, 117]. Thus, conceivably, anti-IL-24 and MMP-7 therapies may be used for oral administration in the therapy of advanced SCC. Taladegib is an experimental SMO homologue antagonist which is being investigated in clinical and preclinical stages. Its clinical responses were shown to be lower in comparison to the previous SMO inhibitors so far [5]. However, it was shown to inhibit the activity of vismodegib-resistant SMO homologue mutant (D473H), emphasizing its clinical importance [118]. Saridegib is another SMO homologue antagonist drug that is currently starting to be investigated in humans [118]. More studies should be performed to gain sufficient knowledge about their efficacies in these pathologies.

Itraconazole, generally known as a potent antifungal agent, has shown evidence as a powerful inhibitor of the hedgehog pathway, inhibiting the growth of BCC. Its mode of action differs from other SMO homologue antagonists since it enables the prevention of SMO homologue localization to cilia. Thus, it is known as cilial translocation inhibitor. Only short-term clinical trials were carried out with insufficient and inconclusive outcomes by this time. Itraconazole administration did not allow the complete disappearance of the tumor, suggesting, build on these achievements, that itraconazole treatment for BCC is inferior to vismodegib. Moreover, the efficacy of itraconazole was shown to be dose dependent, assuring the high-dose long-term treatment to meet with the efficacy achieved by other hedgehog pathway inhibitors, as vismodegib and sonidegib [64]. On the other hand, itraconazole was not specifically studied for any of the locally advanced or metastatic BCC, being only generally used for BCC. Thus, further larger clinical trials need to be carried out to furnish clearer outcomes on the administration of itraconazole in BCC cases. The investigation of its particular mode of action is particularly relevant, since it could be an important target in cases resistant to other hedgehog inhibitors considering its different binding interactions.

There is a growing body of evidence suggesting cross talk between the hedgehog pathway and other signaling pathways in the initiation and progression of BCC. Besides the promising results of all hedgehog pathway inhibitors, their failure is also evident for resistant tumors limiting their use [63, 64]. Alternative strategies, as combined therapies, are needed to overcome the drug resistance of distinct advanced BCCs. Currently, there are several combinational clinical trials in progress using hedgehog pathway inhibitors. The PI3K-AKT-mTOR pathway is often activated in carcinogenic processes, and thus PI3K is an attractive target for cancer therapy. Notwithstanding, the PI3K pathway is also involved in resistance to anticancer therapies, comprising chemotherapy, radiotherapy, hormonal therapy, and targeted drugs. Pictilisib, copanlisib, taselisib, alpelisib, and idelalisib are PI3K inhibitors which show promising preliminary antitumor properties and acceptable safety profiles in subjects with recurrent metastatic head-and-neck SCC. These agents may restitute sensitivity to other treatments when administered as part of combination therapeutics. Clinical trials using PI3K inhibitors are under way in combination with other drugs, as paclitaxel [119].

In an attempt to obtain more potent and orally available hedgehog pathway inhibitors, recent research showed that several novel therapies containing trifluoromethyl 4-(2-pyrimidinylamino)benzamide derivatives were found to display more potent inhibitory activity to hedgehog pathway signaling than vismodegib [120]. These compounds are thus suggested as relevant compounds for further studies.

Fibroblast growth factor receptor (FGFR) inhibitors are promising candidates for targeted therapeutics in multiple types of solid tumors, including head-and-neck SCC. This is particularly important since the use of targeted therapies for the treatment of advanced head-and-neck SCCs is confined to the EGFR inhibitor cetuximab. Treatment of an FGFR1-overexpressing head-and-neck SCC cell line with the potent and selective pan-FGFR inhibitor AZD4547 was found to reduce cell proliferation and FGFR signaling. Besides, EGFR signaling was distinguished as a resistance mechanism for FGFR inhibition, which can be overpassed by combined AZD4547 and gefitinib treatment. Clinical trials on AZD4547 are already being carried out [121].

Furthermore, wild-type and mutated FGFR3 was evaluated newly as a possible therapeutic target in head-and-neck SCCs. Authors found that FGFR3 executes a relevant role in tumor cell proliferation, although its expression is diminished throughout head-and-neck SCC evolution. Further clinical trials are demanded to check the possible positive results of specific new FGFR3 inhibitors in the treatment of this clinical condition [122].

Lysyl oxidases (LOXs) are a family of copper-dependent amine oxidases expressed in various human tumors. LOX-like 4 (LOXL4), a new member of the LOX group, was found to be overexpressed in head-and-neck SCCs. Thus, the fusion of Balb/c mouse splenocytes immunized with LOXL4-specific peptide gave origin to a monoclonal antibody which exhibited tumor-specific upregulation of LOXL4. These data foster the investigation of the antitumor targeting activity of this potential therapeutic immune agent in the treatment of head-and-neck SCCs [123].

Over the past 2 decades, the rapid developments in nanotechnology have allowed the incorporation of chemotherapeutic agents into nanoparticles to prevent and treat skin cancer, including NMSCs. Nanocarriers seem to be suitable to overcome the heterogeneity of cancer cells and multiple drug resistance, and to enhance the intracellular concentration of chemotherapy agents and consequently their cytotoxicity [124, 125]. However, nanocarriers should be very well characterized, especially their interaction with biological components, i.e., the assessment of nanotoxicology should be very well established.

Intralesional chemotherapy (direct therapeutic injection into a lesion) is seldom used for NMSC, but it remains an option for well-selected patients who cannot or will not undergo surgery. Intralesional therapies nowadays include: bleomycin, 5-FU, methotrexate, and various interferons, but most studies are small case series with fewer than 20 patients [14].

Electroporation-based therapies are progressively being used in cancer clinical research due to their safety and efficiency in the transfer of a wide range of materials (as nucleic acids, cytotoxic drugs, and ions) into target cells and tissues. The use of electroporation to enhance anticancer drug uptake is called electrochemotherapy. This is an effective local anticancer treatment exploited for superficial lesions and it is being assessed for deep-seated lesions. It is increasingly used in the palliative treatment of cutaneous and subcutaneous cancerous nodules. Due to its feasibility and safety, efforts are being gathered to transfer this local antitumor treatment into a systemic one.

Immunotherapy

Electrogenetherapy is the electroporation-mediated transfer of therapeutic genes into the cell. It is a very effective and safe tumor-debulking approach showing immune-stimulating characteristics through immunogenic cell death stimulation. Particularly, skin is prone for vaccination as it is rich in antigen-presenting cells, such as dendritic cells. DNA vaccination and cytokine-based immunotherapy constitute electrogenetherapy approaches that address antitumor immunity. Preclinical evidence indicates that the combination of electrochemotherapy with immune-stimulating electrogenetherapy (by the administration of immune-stimulating agents, e.g., antitumor cytokines) could be an encouraging approach to treat both accessible electrochemotherapy-treated nodules and any systemic indiscernible metastasis. So, recently, local electrochemotherapy of skin melanoma lesions combined with ipilimumab led to total regression of all cutaneous and visceral metastases. In addition, clinical studies using electrogenetherapy are ongoing in subjects with head-and-neck SCCs. In another study, the authors treated SCCs concomitantly with electrochemotherapy and intratumoral IL-12 gene electrotransfer. This study supported an enhanced efficacy, inhibition of metastasis evolution, and prolonged survival in relation to the single use of each treatment. This way, the association of elec-

trochemotherapy with immune stimulation is a very hopeful way for complete and long-term cancer eradication. Further understanding is urgently demanded to authenticate the strong therapeutic potential of the previous combinations [126–128].

Conclusions

Additional research into tumorigenesis and the molecular biology of NMSCs is needed. This could yield the discovery of new pathways for targeted oral therapies developed in order to treat advanced forms of these increasingly common malignancies. Moreover, a larger knowledge of resistance mechanisms will enable the rational design of combination treatments and sequential therapy algorithms to improve clinical outcomes. Treatment alternatives should be stratified based on patient risk of advanced disease/failure of classical treatment associated with the adverse effects of intravenous chemotherapy. Knowledge of the molecular biology of NMSCs and tumorigenesis, looking into tumor biomarker profiles, will potentially pave new pathways for targeted therapies of advanced types of these progressively frequent malignancies. These are recommended for the accurate prediction of the patients' response to specific treatments. A case is made towards the use of targeted hedgehog inhibitors for advanced BCC and neoadjuvant use of EGFR inhibitors for advanced SCC. For the treatment of advanced SCC, evidence exists for adjuvant oral retinoids, capecitabine, and 5-FU, but for high-risk groups (e.g., immunosuppressed patients) data are in favor of chemoprevention. Nowadays, a correlation between the circadian rest-activity cycle and several chronopharmacology mechanisms present in some species has been the basis for the chronotherapy currently under study in cancer patients.

Acknowledgments

The authors would like to acknowledge the financial support received from the Portuguese Science and Technology Foundation (FCT/MCT) and from European Funds (PRODER/COMPETE) under the project references M-ERA-NET/0004/2015-PAIRED and UID/QUI/50006/2013, cofinanced by FEDER, under the Partnership Agreement PT2020. The authors also wish to acknowledge FCT for the financial support of the individual scholarship (SFRH/BD/109261/2015). The Spanish Ministry of Science and Technology is also acknowledged under the reference MAT 2014-59134-R.

Disclosure Statement

The authors report no conflict of interests with respect to this work.

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