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The genetics of cutaneous squamous cell carcinogenesis

In this review, the current knowledge of cutaneous squamous cell carcinogenesis (cSCC) is outlined based on an appraisal of the different features of cSCC, with particular emphasis on genetic alterations underlying aetiopathogenesis. When appropriate, diagnostic and/or prognostic biomarkers for cSCC are also considered. This review is intended to aid future investigation into the molecular characterization of cSCC.

Key words: carcinogenesis, cutaneous squamous cell carcinoma, cSCC, genetics

Cutaneous squamous cell carcinoma (cSCC) is the second most common cancer in Caucasians with an incidence of about one million cases per year [1]. Recent population studies report that age-standardized incidence rates are rapidly rising with absolute increases of approximately 2,000 new cSCC cases annually in countries with 4.5 to 9 million inhabitants [2-4]. Ultraviolet radiation is the most common causal factor [5]. Other risk factors include fair skin, blue eyes, a history of sunburn during childhood, exposure to ionizing radiation, genodermatosis, organ transplants (with a 65-fold increased risk), and chronically injured or diseased skin. Although the effect of tobacco is not as great as for other SCC, tobacco may double the risk of cSCC [6]. cSCC follows a classic multistep carcinogenesis model: premalignant lesion (actinic keratosis), *in situ* squamous carcinoma/Bowen disease, invasive carcinoma, and metastatic carcinoma. Patients with multiple actinic keratosis (AK) have a 6-10% life-time risk of cSCC [7] and the estimated rate of progression to cSCC for a single AK is reported at 0.025-16% (per year) [8]. Some studies state that 65% cSCC cases arise from AK [9]. cSCC can recur (3-5%) and metastasize (4-5%) [10].

Another entity, keratoacanthoma (KA), has generated controversy since its first description in 1889. Discussion between scholars as to whether this entity is benign or malignant, or whether it corresponds to a well-differentiated cSCC or a distinct entity, has been ongoing for decades. Contrary to cSCC, KA is assumed to originate from the hair follicle, which suggests a benign nature [11, 12], but similar to cSCC, UV radiation is the predominant risk factor [12]. Other risk factors include immunosuppression, skin trauma (*e.g.* surgical procedures, chemical peeling, dermabrasion, cryotherapy, photodynamic therapy or irritation after application of tar and imiquimod), and treatment with BRAF inhibitors and Hedgehog pathway inhibitors [13, 14]. Clinically, it may present as a solitary lesion or as multiple lesions (Ferguson-Smith type) [14, 15]. Histologically, architectural differences and immunohistochemical markers make it possible to differentiate between cSCC and KA.

cSCC most frequently occurs in chronically sun-exposed areas, such as the face (particularly the lip, ear, nose, cheek, and eyelid) and the dorsum of the hands. The head and neck are the most affected areas in males, while the upper limbs followed by the head and neck are the most common locations in females. In order to aid prognostic and appropriate management, cSCC cases are classified based on histological subtype (acantholytic, spindle, verrucous, and desmoplastic), grade of differentiation (well-differentiated, moderately differentiated, poorly differentiated or undifferentiated), tumour depth (maximum vertical diameter), level of dermal invasion (Clarkís level), and the presence or not of perineural, lymphatic or vascular invasion [16]. Although not optimal for cSCC, to date, staging is based on the TNM system of the 2010 American Joint Committee on Cancer guidelines [17]. Patients with localized cSCC usually have excellent outcome, but metastatic cSCC has a poor prognosis with a 25-35% five-year survival rate and <10% ten-year survival rate [18]. Prognostic cSCC factors associated with the development of metastasis include recurrence, tumours arising from scars, clinical size (>2 cm), histological type (acantholytic, spindle, and desmoplastic subtypes), tumour thickness (>6 mm), horizontal size, poor differentiation, perineural invasion, subcutaneous fat invasion, immunosuppression, and location on the lip, ear and possibly the temple [10, 19]. The epidemiology of cSCC is summarized in *table 1*.

Notwithstanding recent advances, the molecular profile of cSCC is far from clarified. Compared to other SCC (*e.g.*

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Table 1. Epidemiological features of cSCC.

	cSCC
Incidence	About one million cases per year
Anatomical location	Skin
Risk factors	Ultraviolet radiation, fair skin, blue eyes, a history of sunburns during childhood, exposure to ionizing radiation, certain genodermatosis, organ transplants, chronically injured or diseased skin, tobacco
Protective factors	Sun protection
Precursor lesions	Actinic keratosis
Recurrence rate	3-5%
Rate of metastasis	4-5%
Field cancerization	Yes
5-year survival rate	Localized: excellent Metastasized: 25-35%

lung, head and neck), there is little information about the molecular genetics of cSCC. With this in mind, we sought to review the reported molecular alterations in cSCC in a comprehensive way, in order to aid future investigation. In the following review, for the sake of simplicity, the molecular and genetic alterations are considered according to the main molecular anomalies associated with the development of cSCC.

Cell cycle regulation and apoptosis

TP53

The tumour suppressor protein most frequently inactivated in cSCC is p53, encoded by the gene *TP53*, known as the "guardian of the genome" [20]. p53 is a tumour suppressor protein which contains transcriptional activation, DNA binding, and oligomerization domains. This protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Anomalies associated with these events often result from mutation of *TP53* but can also occur as a result of interactions between p53 and viral proteins such as HPV E6 [14].

The molecular abnormalities in cSCC are summarized in *table 2* and *figure 1*.

TP53 tumour suppressor gene mutation is the most common and the earliest identified genetic alteration in cSCC. Mutations occur in up to 90% of cSCC cases but less in premalignant lesions (7-48%). The reported variability of the mutation rate in AK is suggested to be due, in part, to the different severity of the lesions investigated [21, 22]. *TP53* mutation seems to be frequent in metastatic cSCC (79%; 24/29 cases) [23]. A more recent targeted sequencing study demonstrated a significantly higher mutation frequency in metastatic tumours compared to primary tumours (85% vs 54%; $p < 0.002$), highlighting the importance of functional p53 as a barrier to cancer progression [24]. Nevertheless, the prognostic impact of these mutations requires clarification.

Retinoblastoma

The retinoblastoma gene (*RB1*) is another major tumour suppressor gene involved in cell cycle regulation. RB1 protein stabilizes constitutive heterochromatin to maintain the overall chromatin structure, and the active hypophosphorylated form of the protein binds transcription factor E2F1 [14] to control gene transcription.

There are few studies on cSCC reporting *RB1* inactivation or RB1 protein loss. In one of the few immunohistochemical (IHC) studies, loss of expression was reported in 8% (2/26) of AK and 16% (7/45) of cSCC cases [25].

Cyclin D1

Cyclin D1 (*CCND1*) accelerates the passage of cells through G1 phase and reduces the requirement for mitogens [17]. This protein is described to participate in tissue organization and differentiation in the early stages of cSCC [26], with overexpression frequently reported in keratinocyte carcinogenesis [25, 27]. One study reported cyclin D1 overexpression in 46% (12/26) of AK and 60% (27/45) of cSCC cases [25]. Another study reported overexpression in 43% (13/30) of Bowen Disease (BD) and 71% (17/24) of cSCC cases [28]. The overexpression of cyclin D1 in premalignant lesions (AK) suggests that it may be an early event in cSCC carcinogenesis. Although studies have reported an increase in expression with increasing histological differentiation in oral SCC, there is a lack of correlation between cyclin D1 overexpression and the degree of differentiation in cSCC [28-30]. One study reported a positive correlation between cyclin D1 overexpression and depth of invasion and metastasis [31]. Larger studies are needed to confirm the prognostic importance of cyclin D1.

Cyclin-dependent kinase inhibitors

These cell cycle inhibitors belong to two main families: the ink4 family (*e.g.* p16 [*CDKN2A*]) and the Cip/Kip family (*e.g.* p21 [*CDKN1A*] and p27 [*CDKN1B*]).

p16 is a specific inhibitor of cyclin-dependent kinases 4 and 6 (*CDK4* and *CDK6*, respectively) [18]. Studies targeting p16 in cSCC are scarce. There are reports of *CDKN2A* alterations (*e.g.* mutations, copy loss, promoter methylation) in 76% cSCC cases and *CDKN2A* mutations in 48% of metastatic cSCC cases [23]. In a study of metastatic cSCC, with a *CDKN2A* mutation frequency of 31% (11/35), *CDKN2A* mutation was associated with disease-specific death ($p = 0.001$) [32]. A more recent study demonstrated a lower rate of mutation (17%) compared to previous studies [24]. Larger studies are needed to confirm p16 as a prognostic biomarker.

To our knowledge, no study has determined the prevalence of overexpression and the mutation rate of Cip/Kip family genes in cSCC.

KNSTRN

KNSTRN encodes the kinetochore localized Astrin/SPAG5 binding protein that assists kinetochore formation during cellular division [19]. In one study, *KNSTRN* mutation was reported in 13% AK and 19% cSCC cases. UV-induced mutation is assumed to occur in premalignant lesions, suggesting that the mutation might be an early event in the

Table 2. Molecular abnormalities in cSCC.

	cSCC		
	Mutations	**Over-expression**	**Down-regulation**
Cell cycle regulation and apoptosis			
TP53	Up to 90%		
RB1			16%
CCND1		60-71%	
CDKN2A	17-48% (metastatic cases)		76%
KNSTRN	19%		
Non-coding promoter mutations			
TERTp	Up to 50%		
Terminal differentiation			
NOTCH1	Up to 82%		
FBXW7			
TP63			
RIPK4	24% (metastatic cases)		
EGFR and other TKRs			
EGFR	2.5-3%	Up to 73%	
PIK3CA	10%		
HRAS	11-13%		
KRAS	10 %		
RASA1	13%		
Adhesion, invasion, and microenvironment molecules			
CDH1			85%
FAT1	44%		

development of cSCC [33]. Still, these results were not confirmed based on a large series of cSCC. *KNSTRN* mutation was also not observed in other studies [23, 24]. Since *KNSTRN* mutations may represent a potential target for new drugs, more studies are necessary to clarify the prevalence and role of *KNSTRN* mutation in cSCC.

Non-coding telomerase (TERT) promoter mutations and stemness

Telomerase (*TERT*) is a ribonucleoprotein complex that synthesizes telomeric DNA (TTAGGG hexamers) which is required to maintain telomere length [34]. *TERT* promoter (*TERTp*) mutations increase telomere length and stability, allowing cancer cells to divide and avoid senescence or apoptosis. Lately, recurrent somatic mutations in the *TERTp*, which affect the catalytic subunit of telomerase, have been described in several cancer models (melanomas, basal cell carcinomas, squamous cell carcinomas, cancers of the central nervous system, and bladder and thyroid cancers [follicular cell-derived]) [35-40].

Some studies have reported *TERTp* mutations in SCC at different locations. Scott *et al*. reported *TERTp* mutation in 50% (13/26) of cSCC and 20% (11/55) of BD cases [38]. *TERT*p mutations were more frequent in cSCC than in BD ($p = 0.019$), suggesting a more relevant role in tumour progression than initiation [38]. Poorly differentiated cSCC is reported to harbour more *TERTp* mutations, but the small size of the series analysed limited the statistical significance of the study [38]. Additional studies have reported *TERTp* mutations in 25-50% of cases [37, 40]. Importantly, in the future, *TERTp* mutations might be used as biological predictors of metastasis and mortality [41], as is the case for melanoma, glioblastoma, medulloblastoma, bladder, and thyroid cancers [40, 42-45]. Larger studies are necessary to ascertain whether *TERTp* mutations have prognostic value for cSCC.

Terminal differentiation factors and retinoid receptors

NOTCH and associated factors

The Notch signalling pathway is involved in the regulation of self-renewal, cell cycle exit, and cell survival [46-48]. Additionally, Notch activity can suppress HPV E6 and E7 protein expression [49].

NOTCH1 and *NOTCH2* mutations have been described in 75% of cSCC cases [49]. Initially, these mutations were reported to occur following homozygous *TP53* mutation, which suggested a more relevant role in tumour progression than initiation [49]. A more recent exome sequencing study reported an 82% mutation rate (with mutation identified in normal skin in 70% cases), identifying *NOTCH1* and *NOTCH2* mutation as an early event in squamous cell carcinogenesis [50].

Based on a whole-exome sequencing study of aggressive cSCC, the frequency of *NOTCH1* and *NOTCH2* mutations appears to be similar (>50%), and more than 30% mutations exhibit loss of function. In another cohort

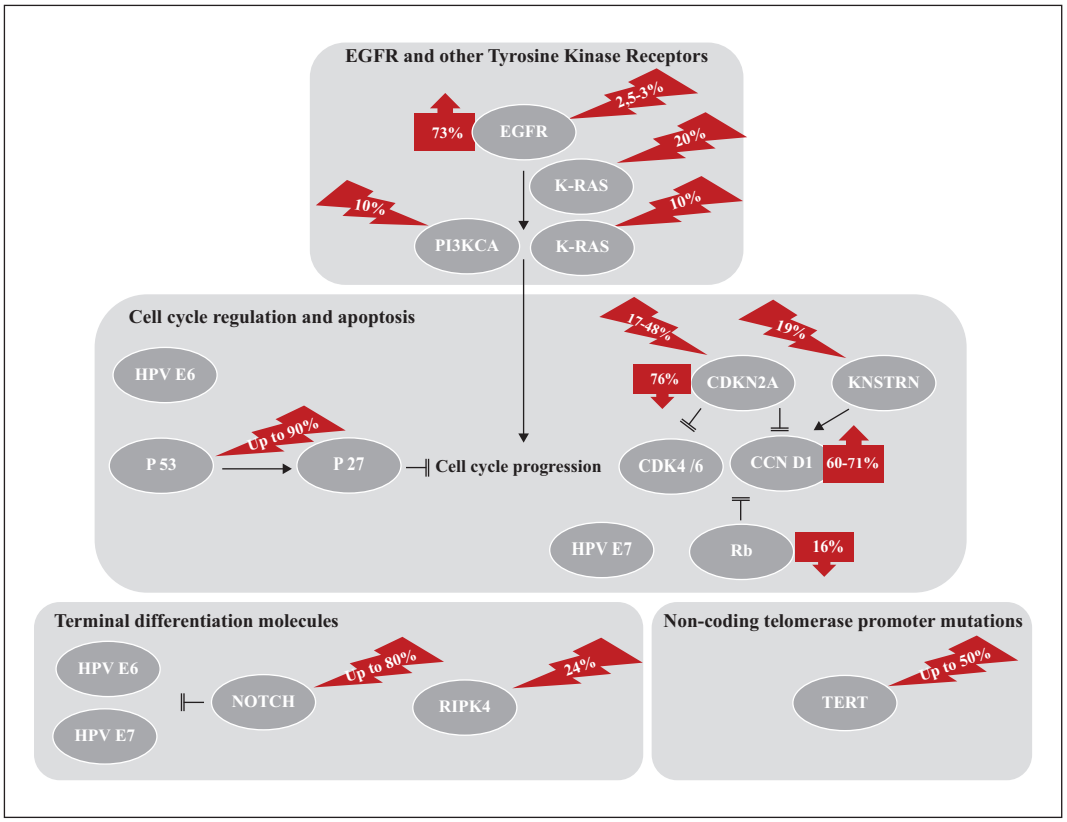

Figure 1. Illustration of the main oncogenic pathways involved in cSCC carcinogenesis. The red lightning symbol indicates the reported incidence of mutations for each oncogene or tumour suppressor gene in cSCC. Upward red arrow indicates overexpression and downward red arrow indicates downregulation in cSCC.

of patients with metastatic cSCC, *NOTCH1/2/4* mutations were reported in 69% of cases [23]; most of the *NOTCH* mutations were missense mutations not previously reported. Another targeted sequencing study of metastatic cSCC reported *NOTCH* mutations in 66% cases [24]. If confirmed, these results would make *NOTCH* mutations the most prevalent genetic alteration in cSCC, however, the frequent identification of these mutations in adjacent normal skin precludes its prognostic value.
A next-generation sequencing study reported that, in addition to *NOTCH1* alterations, *FBXW7* alterations were present in 7% of SCC cases at different locations [51]. *FBXW7* is part of the ubiquitin ligase complex that mediates *NOTCH1* degradation [52], thus constituting an alternative mechanism of *NOTCH* inactivation.

TP63

TP63 is a member of the p53 family and plays a central role in the development of the stratified epithelium, such as the epidermis [53, 54]. This gene may have antagonist roles in cSCC, in contrast to other SCC (*e.g.* head and neck squamous cell carcinoma [HNSCC]). In HNSCC, for example, p63 overexpression is frequent (>95%) and is associated with increased patient survival. On the other hand, p63 expression may be a strong predictor of poor differentiation in non-melanoma skin cancer [55]. In a targeted sequencing study of metastatic cSCC, *TP63* was amplified in 24% (7/29) of cases [23]. However, there are no specific studies addressing *TP63* genetic alterations in cSCC or its putative prognostic value.

RIPK4

An exome-sequencing study identified, for the first time, a potential driver gene in cSCC: *RIPK4* [56]. RIPK4 protein is a serine/threonine protein kinase that interacts with protein kinase C-delta, which is required for keratinocyte differentiation [57]. Based on a genomic analysis of metastatic cSCC, seven cases (24%) with recurrent *RIPK4* mutations were reported [23], two of which were truncating, suggesting recurrent inactivation of the gene. More studies are necessary to ascertain the relevance of *RIPK4* in metastatic cSCC.

Epidermal growth factor receptor and tyrosine kinase receptor pathways

Epidermal growth factor receptor (EGFR) activation or overexpression leads to upstream signalling of both MAPK and PI3K pathways, and is involved in proliferation and evasion of apoptosis [58].
EGFR-activating mutations are rare and have been reported in 2.5-3% of cSCC cases [59, 60]. On the other hand, EGFR

overexpression is described in 43% (9/21) to 73% (30/41) of cSCC cases [61, 62]. EGFR inhibitors (*i.e.* erlotinib and getinib) and EGFR antibodies (*i.e*. cetuximab and panitumumab) are widely used for lung SCC and a need to refine subsets of advanced cSCC that are likely to respond to EGFR therapy is needed. Future research is therefore necessary to clarify this premise.

PI3K pathway

PIK3CA encodes a positive regulator of the PI3K signalling pathway. PI3K is a lipid kinase that converts plasma membrane PIP2 to PIP3 [63] and activates multiple cellular pathways, namely mTOR.
In contrast to other SCC (*i.e*. HNSCC), PI3K pathway mutations do not appear to have a relevant role in cSCC carcinogenesis. Based on an exome sequencing study of cSCC, 10% of cSCC cases presented with *PIK3CA* mutation. These mutations included two inactivating mutations but so far no mutations have been found within the classic hotspot (E545, H1047) [56]. Based on an exome-targeted analysis of metastatic cSCC, oncogenic activation of the RAS/RTK/PI3K pathway was reported in 45% cases and significantly correlated with worse progression-free survival [23]. Although this pathway appears to be important in HNSCC and lung squamous cell carcinoma (LSCC), its role in cSCC is not yet established [56, 64].
In cSCC, no mutations were reported in *PTEN*, which encodes a negative regulator of the PI3K signalling pathway and switches PIP3 to PIP2 [63].

MAPK pathway

RAS oncogenes play a role in different cellular processes (the RAS family controls cell growth and the RHO family controls the actin cytoskeleton). Three members of the RAS family (*HRAS*, *KRAS* and *NRAS*) are reported to be frequently mutated in human tumours [65].
RAS mutations appear to be rare in cSCC. *KRAS* mutation has been reported in 10% cSCC cases [66]. Exome-level sequencing of eight primary cSCC revealed mutation in *HRAS* in one case (13%) [64]. Another exome sequencing study reported an overall activating *RAS* mutation frequency of 11% [50]. Nevertheless, an increased level of RAS with active GTP was described in cSCC, suggesting the possibility that RAS activation in cSCC may also result from upstream stimulation (tyrosine kinase receptor activation), as reported in breast carcinoma [59]. In a cohort of patients with metastatic cSCC, oncogenic activation of the RTK/RAS/PI3K pathway was reported in 45% of cases and significantly correlated with worse progression free-survival [23]. *BRAF* gene mutations are rare events in cSCC [67].
It is important to mention the paradoxical effect observed in melanoma patients treated with tyrosine kinase inhibitors (TKI) concerning the increase in mutated *RAS* in cSCC. In fact, melanoma patients treated with RAF inhibitors develop keratoacanthomas (KA) or cSCC in up to 25% cases [68, 69]. The potential mechanism consists of paradoxical increase in MAPK signalling within the context of mutated or activated *RAS*. Tumours from a cohort of patients treated with a RAF inhibitor were prone to *RAS* mutations despite similar rates of total mutations in patients treated with non-RAF inhibitors [70]. These findings suggest that development of TKI-induced cSCC is not due to a direct mutagenic event associated with RAF inhibitor therapy, but rather due, at least in part, to pro-proliferative interaction between RAF inhibitors and latent *RAS* mutant keratinocytes.

RASA1

RASA1 belongs to a family of RAS GTPase activating proteins, many of which appear to be implicated as tumour suppressors in cancer because they function as negative regulators of the pro-oncogene *RAS* [71]. The role of *RASA1* in cancer has not been clearly defined, despite its frequent inactivation by mutation in many tumour types [72].
Based on an exome sequencing study of cSCC, *RASA1* mutation was reported in 13% of cases [56].

Adhesion, invasion and microenvironmental factors

E-cadherin complex

E-cadherin (*CDH1*) and catenins are key proteins of the adhesion complex at adherent junctions that link neighbouring epithelial cells [73].
There are reports of E-cadherin promoter hypermethylation in 6/7 (85%) cSCC, 4/8 (50%) *in situ* cSCC, 4/9 (44%) AK, and 2/9 (22%) non-neoplastic skin cases. In non-melanoma skin cancer (NMSC), downregulation of E-cadherin is associated with increased tumour invasiveness, an increased potential for distant metastasis, and advanced-stage cSCC [74].

FAT1 gene

The *FAT1* gene is an orthologue of the Drosophila fat gene, which encodes a tumour suppressor essential for controlling cell proliferation during Drosophila development. The gene product is a member of the cadherin superfamily and is expressed at high levels in a number of foetal epithelia [75]. A whole-exome study reported *FAT1* gene mutation in 17/39 (43.6%) aggressive cSCC cases, but without prognostic impact [56].

Matrix metalloproteinases

Matrix metalloproteinases (MMP) are zinc-dependent endopeptidases that can degrade many extracellular matrix proteins [76]. Immunohistochemical expression of MMP2 and MMP9 is associated with cutaneous squamous carcinogenesis and is a potential marker for invasion and progression [77].

Angiogenic and inflammatory factors

Hypoxia leads to an increased production of proangiogenic factors and diminished production of anti-angiogenic factors. Proangiogenic factors include: vascular

endothelial growth factor (*VEGF*), platelet-derived growth factor (*PDGF*), fibroblast growth factors 1 and 2 (*FGF1* and *FGF2*, respectively), and interleukin 8 (*IL-8*), among others [78].
There are reports relating VEGFA overexpression to lymphatic metastasis in mouse models of cSCC [79]. There are no clinical studies relating VEGFA to cSCC prognosis. In cSCC, there appears to be high expression of COX-2 in premalignant and malignant lesions [80], and COX-2 expression increases during progression of the tumour [81].

Other features

Aneuploidy

Aneuploidy, although not a hallmark of malignancy, is more frequent in malignant than benign tumours, and is associated with tumour progression. For cSCC, there are few studies evaluating DNA ploidy, and in some, aneuploidy has been suggested to be significantly associated with a risk of metastasis, however, this association remains to be clarified [82].

Epigenetic alterations

Epigenetic alterations cause modifications in DNA domains involved in the control of gene expression. These alterations include DNA methylation, histone acetylation, phosphorylation, ubiquitination, and sumoylation [83].
In a recent epigenetic study of cSCC, no widespread difference in methylation pattern was reported and *FRZB* was identified as a potential epigenetic predictor of metastasis, however, no significant difference was observed when protein expression was compared between metastatic and non-metastatic cSCC [84]. *KMTC2* is a member of the ASC2/NCOA6 complex (ASCOM) with histone methylation activity and is involved in transcriptional co-activation/regulation. There are reports of inactivating *KMT2C* mutations in several cancers, including leukaemia and carcinomas of the stomach, bladder, and breast. A report on exome sequencing for cSCC described inactivating mutations in *KMT2C* (15/39 [38%] cases); patients with *KMT2C* mutations presented significant shorter periods of recurrent free survival, a shorter time to recurrence, and a trend to develop bone metastasis; these data support a role for *KMT2C* in the aggressive behaviour of cSCC [56]. A more recent targeted sequencing study reported a high mutation rate of epigenetic regulators, such as *KMTD2* (8/12 [67%] cases), *KAT6A* (4/12 [33%] cases), *KMTC2* (7/12 [58%] cases), *SETD2* (6/12 [50%] cases), *ARID2* (2/12 [17%] cases), *TET2* (1/12 [8%] cases), *KDM6A* (1/12 [67%] cases), and *CREBBP* (2/12 [17%] cases) [24]. Larger studies are needed to confirm the prognostic value of these alterations.

Human papillomavirus

Human Papillomavirus (HPV) is a double-stranded DNA virus that infects the squamous epithelium. HPV genotypes are classified into five genera: α, β, γ, μ, and ν, based on the degree of sequence similarity. HPV can be subdivided into low and high risk, depending on the malignant progression potential of the associated lesion. High-risk mucosal HPVs cause almost all cases of cervical cancer, and are also associated with a significant fraction of other anogenital tract cases, as well as oropharyngeal cancers [85]. The mechanism of oncogenesis is ascribed to viral proteins E6 (which binds to p53, rendering it a target for proteasomal degradation) and E7 (which binds to RB1, rendering it a target for proteasomal degradation), leading to a loss of tumour suppressor genes that inhibit cell cycle progression [86].
HPV 5 and 8 have been reported in 90% of cSCC cases as a rare genetic disease termed "epidermodysplasia verruciformis" (EV). The association of HPV 5 and 8 with cSCC in EV patients led to their classification as "possibly carcinogenic" [87]. β HPV are also the likely aetiological agents of cSCC that arises in chronically immunosuppressed patients. The association between HPV infection and cSCC development in immunocompetent patients remains controversial [87]. While β HPV genomes are frequently detected in cSCC specimens, they are also often found on healthy skin of non-EV individuals [88, 89]. Epidemiological studies have demonstrated that the prevalence of β HPV in AK is higher than in cSCC suggesting that β HPV may play a role during the initial stages of carcinogenesis [90, 91]. Despite these initial reports, HPV transcription in cSCC has not been identified in recent high-throughput sequencing studies [92, 93]. A possible explanation resides in the fact that the majority of β HPV E7 and E6 proteins, including those of HPV 5 and 8, do not have the ability to destabilize p53 and RB1.

Keratoacanthoma

Based on array comparative genomic hybridization, it has been possible to successfully discriminate between KA and cSCC in 85% of cases, leading to the assumption that these are two distinct entities [94]. Molecular identification of mutations in *TGF1* (which encodes TGFß) in KA of Ferguson-Smith type (85-90%) and its absence in cSCC suggests the existence of a distinct pathogenic pathway [95, 96]. Despite the identification of *TGF1* mutations, larger studies are required to establish this mutation as an unequivocal molecular marker in KA.

Conclusion and future perspectives

In an era of predictive biomarkers and patients stratified for therapy, in which new drugs with various molecular targets are being developed, a comprehensive understanding of the molecular basis of cSCC is of outstanding importance, especially for patients with metastatic disease in which prognosis is poor and effective therapies are lacking. Despite improvements in surgery, chemotherapy, radiotherapy, and supportive care, overall survival has not markedly improved for patients with advanced cSCC. Current chemotherapy treatments for cSCC are not targeted, but instead primarily platinum-based treatments (cisplatin and carboplatin) with concurrent radiation are used. Options for recurrent/metastatic cSCC remain very limited and despite significant improvements in targeted treatment for other skin cancers (*e.g.* melanoma and basal cell carcinoma), currently there is no targeted therapy approved for cSCC. Our

increasing knowledge of molecular alterations concerning cSCC opens new avenues in the design of more efficient targeted therapies.

Retinoids act on retinoid acid receptors, mediate epidermal growth factor genes, and inhibit dermal microvascular endothelial cells and neutrophil migration. These have been used successfully to prevent the development of cSCC in immunosuppressed patients after renal transplantation [49-51], however, the use of retinoids in chemoprevention of cSCC in immunocompetent patients was not approved by the FDA [52, 53]. New interest in the use of retinoids in chemoprevention has emerged for patients with development of cSCC following treatment with BRAF inhibitors for metastatic melanoma (7-31%) [54]. At this moment in time, no targeted therapy is approved for advanced cSCC. Few case reports have demonstrated that cetuximab presents a better response rate compared to conventional chemotherapy in patients with metastatic cSCC. We believe that the new monoclonal antibodies (transtuzumab, pertuzumab, onartuzumab, and cixutumumab) and tyrosine kinase inhibitors (geftinib, erlotinib, lapatinib, and afatinib), that target EGFR and other members of the EGFR family, may play a role in the therapy of cSCC, but predictive biomarkers in prospective clinical trials are needed [97]. The *PIK3A* gene is mutated in cSCC, making this pathway an attractive target for therapeutic inhibition (developed molecules include GDC-0941, PX-866, NVP-BKM120, and NVPBYL719). *TP53* alterations are present in the majority of cSCC cases, thus the ability to selectively target tumours with decreased p53 activity could have major implications for these patients. Several experimental strategies have been undertaken to target tumour cells, leading to wild-type p53 activation and restoration (*e.g.* RITA, nutlins, mdm2-inhibitors, and benzodiazepinedione) or mutant p53 reactivation (CDB3, c-terminal peptides, and CP-31398) [98]. *In vitro* studies have suggested that NOTCH inhibitors (gamma-secretase inhibitors and monoclonal antibodies) may play a role in cancer treatment, although no study has so far included cSCC [99]. TERT-targeted therapies (*e.g.* GRNI63, T-oligo, DN-hTERT, BIBRI532, BRACOI9, RHPS4, and telomestatin) are a promising treatment option in cSCC, since a majority of the tumours present with *TERTp* mutations. However, the clinical testing of some of these molecules has been hampered due to the toxic characteristics of the drugs [39]. Overexpression of programmed cell death protein 1/programmed death-ligand 1 (*PD-1/PD-L1*) has been described in cSCC [100]. In this context, immune checkpoint antibodies (*e.g.* ipilimumab, pembrolizmumab, and nivolumab) that block the *PD-1/PD-L1* pathway have been reported in advanced unresectable or metastatic cSCC [101-105]. Patients treated with these anti-*PD-1* inhibitors showed a partial response with a favourable side-effect profile, suggesting that these treatments may represent a promising new therapeutic option for advanced-stage cSCC.

We believe that the next few years will reveal a development of biologic therapies which efficiently target these genetic alterations and improve the survival of patients with cSCC. ■

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