Cutaneous Squamous Cell Carcinoma Arising in Immunosuppressed Patients: A Systematic Review of Tumor Profiling Studies

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As solid organ transplantation becomes more prevalent, more individuals are living as members of the immunosuppressed population with an elevated risk for cutaneous squamous cell carcinoma (cSCC). Although great progress has been made in understanding the pathogenesis of cSCC in general, little is known about the drivers of tumorigenesis in immunosuppressed patients and organ-transplant recipients, specifically. This systematic review sought to synthesize information regarding the genetic and epigenetic alterations as well as changes in protein and mRNA expression that place this growing population at risk for cSCC, influence treatment response, and promote tumor aggressiveness. This review will provide investigators with a framework to identify future areas of investigation and clinicians with additional insight into how to best manage these patients.

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INTRODUCTION

Over 5 million cases of nonmelanoma skin cancer (NMSC) are diagnosed each year in the United States, which is more than all other cancers combined (American Cancer Society, 2019; Garrett et al., 2017; Madan et al., 2010). Cutaneous squamous cell carcinoma (cSCC) accounts for 20−30% of all NMSC and leads to local recurrence and metastasis at rates of 10% and 3−5%, respectively (Garrett et al., 2017). cSCC is estimated to be responsible for up to 9,000 deaths annually in the United States (Karia et al., 2013).

cSCC is the most common post-transplant malignancy in organ-transplant recipients (OTRs) (Garrett et al., 2017). The number of organ transplants performed in the United States has tripled in the last 30 years, with over 36,000 transplants performed in 2018 compared with 12,623 performed in 1988 (United States Department of Health & Human Services, 2021). Owing to advances in immunosuppressive drug regimens and medical care, patients are now living longer lives post-transplant. Kidney transplant recipients live an average of 8−20 years after their transplant, and 50% of liver transplant recipients are alive after 20 years (Beth Israel Deaconess Medical Center Transplant Institute, 2018; Petrowsky et al., 2013). Transplant patients are 65−108 times more likely to develop cSCC than the general population, and the morbidity ratio in OTRs with cSCC increases by 60−250 times compared with immunocompetent patients (ICPs) (Euvrard et al., 2003; Jensen et al., 1999; Lindelöf et al., 2000; Moloney et al., 2006; Tessari et al., 2010). These differences have been attributed to a more aggressive phenotype, a higher risk of metastasis (approximately 7%), and an increased recurrence rate (7−45%) (Berg and Otley, 2002; Lanz et al., 2019; Sheil et al., 1993).

Genetic and epigenetic alterations between cSCC arising in OTRs and ICPs may contribute to the differences in the behavior of cSCC in these groups. In cSCC in ICPs, classic mutational signatures exist, such as mutations in *NOTCH1/2/4*, *CDKN2A*, and *P53* (Li et al., 2015; Lobl et al., 2021). These mutations may also be present in the OTR population, but the prevalence of these mutations in the OTR population remains to be elucidated. In addition, gene mutations may be influenced by immunosuppressive therapies used to prevent transplant rejection, which may lead to varying mutational signatures even among OTRs (Harwood et al., 2017). cSCC occurring in patients on azathioprine (AZA), for example, are more likely to have the classic mutational signature of *NOTCH1/2/4*, *CDKN2A*, and *P53*, as described above, than patients on other immunosuppressants (Inman et al., 2018). Few reports exist in the literature that compare differences in cSCC between the OTR and ICP populations, and a complete systematic review has yet to be performed. This report aims to provide a complete summary and analysis of reports in the literature regarding the genetic and epigenetic alterations, polymorphisms (germline mutations found in every cell of the

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Abbreviations: 6-TG, 6-thioguanine; AZA, azathioprine; CNI, calcineurin inhibitor; CsA, cyclosporine; cSCC, cutaneous squamous cell carcinoma; hBD, human β-defensin; ICP, immunocompetent patient; ISP, immunosuppressed patient; miRNA, microRNA; NMSC, nonmelanoma skin cancer; OTR, organ-transplant recipient; p-Smad, phosphorylated Smad; RTR, renal transplant recipient; SCC, squamous cell carcinoma; STAT, signal transducer and activator of transcription; TAK1, TGFβ-activated kinase 1; VNO, voriconazole-N-oxide

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body), and changes in protein and mRNA expression in cSCC arising in immunosuppressed patients (ISPs) compared with ICPs and ISPs with and without cSCC. A better understanding of the differences in cSCC between these two populations may further facilitate meaningful research investigation and improve patient management.

RESULTS

Search results

Databases were queried for all articles indexed before 1 November 2021 with no start date, and a total of 2,594 articles were retrieved from MEDLINE, Cinahl, and Scopus (Figure 1). An additional six articles were identified during the review and were also included. After removing duplicates, 2,138 articles remained. A total of 1,979 articles were excluded after reviewing title and abstract. A full-text assessment of the remaining 159 articles was completed and a further 81 articles were excluded for not meeting the inclusion and exclusion criteria, resulting in 78 articles for the qualitative synthesis (Figure 1). The characteristics of original articles included are provided in the Tables 1–8

Study populations

The majority of the compiled articles focused on renal transplant recipients (RTRs) (30 of 78 studies) and the general organ-transplant population (26 of 78 studies). A slightly smaller number of studies focused on cell and mouse models (17 of 78 studies), and even less on nonspecified immunosuppression (7 of 78 studies) and other specified organ transplants including the heart, liver, and lung (3 of 79 studies) (Tables 1–8).

Protein and mRNA expression

There were 30 studies (38% of all articles) that examined protein and mRNA expression and a total of 63 unique proteins/genes of interest (Tables 1 and 2). Of these, 21 studies (27% of all articles) directly compared primary cSCC tumors in ICPs and their immunosuppressed counterparts, with a total of 50 unique proteins/genes of interest being identified (Table 1). Genes with significantly higher expression in cSCC from ISPs than in ICPs included markers of growth and proliferation *FOXO1* and *MKI67* ($P = 0.007$ and $P < 0.05$, respectively) (Feldmeyer et al., 2016; Zhang et al., 2013). Conversely, levels of the activated oncogene

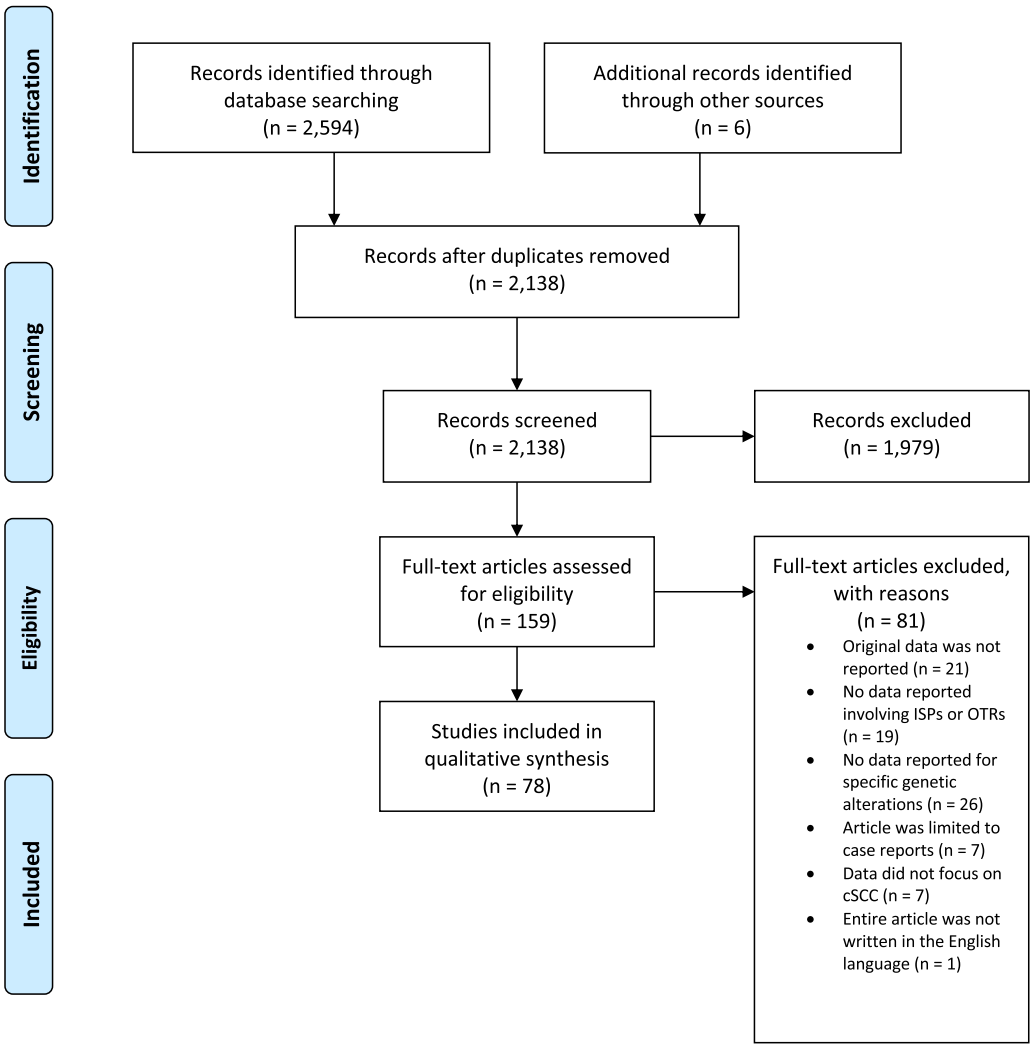

Figure 1. PRISMA 2009 flow diagram. PRISMA flowchart depicting steps taken during systematic review of the literature for the genetic alterations in ISPs and OTRs. From: Moher et al. (2009). cSCC, cutaneous squamous cell carcinoma; ISP, immunosuppressed patient; OTR, organ transplant recipient;. PRISMA, preferred reporting items for systematic reviews and meta-analyses.

Table 1. Gene Expression Differences in cSCC between ISPs and ICPs

Proteins/Genes of interest	Author	Year	Study Population	Findings
B7-H3	Varki et al.	2018	SCC from 42 ICPs and 24 ISPs (13 OTRs, 8 HIV, and 3 others)	60% of ICP tumors expressed B7-H3 in comparison to 28% of ISP tumors ($P = 0.025$).
Bax	Seçkin et al.	2002	SCC from 10 RTRs and 14 ICPs	No difference in Bax expression ($P > 0.05$).
Bcl-2	Seçkin et al.	2002	SCC from 10 RTRs and 14 ICPs	No difference in Bcl-2 expression ($P > 0.05$).
Bcl-xL	Burke et al.	2015	SCC from 21 RTRs and 10 ICPs	Decreased Bcl-xL expression staining intensity was found in RTRs when compared with ICPs (1+ [48%], 2+ [52%], and 3+ [0%] vs. 1+ [10%], 2+ [80%], and 3+ [10%], respectively; $P = 0.042$).
CD4	Zhang et al.	2013	SCC from 12 OTRs and 20 ICPs	OTRs had decreased numbers of CD4+ Th1 T cells compared with ICPs (15.1% $\pm$ 2.3% vs. 25.1% $\pm$ 3.2%, respectively; $P < 0.05$).
CD8	Carroll et al.	2010	SCC from 25 RTRs and 25 ICPs	Ratio of CD8-to-FOXP3 expression was significantly lower in SCC excised from RTRs than in matched SCC from ICPs (1.4 vs. 2.1, respectively; $P = 0.013$).
	Frazzette et al.	2020	SCC from six OTRs and five ICPs	Increased numbers (>6-fold) of primary cSCC tumors were noted in OTRs who as a group showed decreased CD8+ T-effector cells.
Fas	Seçkin et al.	2002	SCC from 10 RTRs and 14 ICPs	No difference in Fas expression ($P > 0.05$).
FOXO1	Feldmeyer et al.	2016	SCC from 10 OTRs and 7 ICPs	Four-fold higher expression of FOXO1 in OTRs than in ICPs ($P = 0.007$).
FOXP3	Zhang et al.	2013	SCC from 12 OTRs and 20 ICPs	Proportion of FOXP3+ cells to CD8+ cells was increased in OTRs compared with ICPs by nearly two-fold (0.97 $\pm$ 0.22 vs. 0.45 $\pm$ 0.05, respectively; $P < 0.05$).
	Carroll et al.	2010	SCC from 65 RTRs and 51 ICPs	Ratio of CD8-to-FOXP3 expression was significantly lower in SCC excised from RTRs than in matched SCC from ICPs (1.4 vs. 2.1, respectively; $P = 0.013$).
	Kosmidis et al.	2010	SCC from 42 OTRs and 43 ICPs	FOXP3 mRNA and protein expression was diminished in OTRs compared with ICPs (by qPCR: $P = 0.045$; OTR mean 6.89×10^{-4}, SD $\pm$ 6.64×10^{-4}, interquartile range 1.35×10^{-4} to 1.2×10^{-3}; ICP mean 2.44×10^{-3}, SD $\pm$ 4.96×10^{-3}, interquartile range 4.6×10^{-4} to 1.74×10^{-3}; and by IHC: $P = 0.04$).
GATA-3	Kosmidis et al.	2010	SCC from 42 OTRs and 43 ICPs	GATA-3 expression did not differ with immunosuppression ($P = 0.08$; OTR mean 1.89×10^{-2}, SD $\pm$ 1.01×10^{-2}, interquartile range 1.03×10^{-2} to 2.75×10^{-2}; ICP mean 1.15×10^{-2}, SD $\pm$ 1.02×10^{-2}, interquartile range 3.08×10^{-3} to 1.74×10^{-2}).
hBD1	Muehleisen et al.	2012	SCC from 11 OTRs and 17 ICPs	SCC in OTRs, in contrast to ICPs, did not overexpress hBD1 in tumors compared with normal skin (ICP difference: $P < 0.05$; OTR difference $P > 0.05$).
hBD2	Muehleisen et al.	2012	SCC from 11 OTRs and 17 ICPs	hBD2 showed increased expression in both OTRs and ICPs compared with normal skin (OTR difference: $P < 0.01$; ICP difference: $P < 0.01$).
hTERT	Perrem et al.	2007	66 RTRs and 66 ICPs (44 SCC and 22 BD in each)	Larger percentage of SCC RTR tumors (18 of 39; 46.2%) had higher hTERT expression than ICP tumors (12 of 42; 28.5%) but not statistically significant ($P = 0.1738$).
IFN-γ	Kosmidis et al.	2010	SCC from 42 OTRs and 43 ICPs	*IFNγ* mRNA expression was decreased in OTRs compared with ICPs ($P = 0.02$; OTR mean 1.56×10^{-4}, SD $\pm$ 2.87×10^{-4}, interquartile range 2.1×10^{-5} to 1.52×10^{-4}; ICP mean 5.65×10^{-4}, SD $\pm$ 8.3×10^{-4}, interquartile range 8.63×10^{-5} to 8.2×10^{-4}).
IL-17A	Kosmidis et al.	2010	SCC from 42 OTRs and 43 ICPs	*IL17A* mRNA was decreased in OTRs compared with ICPs ($P = 0.016$; OTR mean 5.03×10^{-5}, SD $\pm$ 5.18×10^{-5}, interquartile range 1.33×10^{-5} to 7.43×10^{-5}; ICP mean 6.78×10^{-4}, SD $\pm$ 9.4×10^{-4}, interquartile range 5.19×10^{-5} to 1.19×10^{-3}).
IL-22	Zhang et al.	2013	SCC from 12 OTRs and 20 ICPs	Mean *IL22* mRNA expression was increased approximately 30-fold in ICPs and 8-fold in OTRs compared with normal skin ($P < 0.05$). *IL22* mRNA was increased 111-fold in ICP and 97-fold in OTR peritumoral skin.
Ki-67	Zhang et al.	2013	SCC from 12 OTRs and 20 ICPs	Ki-67 expression increased nearly two-fold in OTRs when compared with ICPs (55.08 $\pm$ 7.3 cells/μm^2 $\times$ 10^5 vs. 30.12 $\pm$ 7.1 cells/μm^2 $\times$ 10^5 mean $\pm$ SEM, respectively; $P < 0.05$). Ki-67 expression pattern in OTR tumors was diffused, whereas in ICP tumors expression was mostly present on the periphery of tumor nests.
MAGE-A4	Muehleisen et al.	2007	SCC from seven OTRs and nine ICPs	OTRs showed scattered expression of MAGE-A4, whereas ICPs showed focal expression (3 of 4 (75%) vs. 4 of 4 (100%), respectively).
Mcl-1	Burke et al.	2015	SCC from 21 RTRs and 10 ICPs	No difference existed between groups in Mcl-1 expression intensity ($P = 0.277$) or percentage of cells with Mcl-1 expression ($P = 1.00$).

(*continued*)

Table 1. Continued

Proteins/Genes of interest	Author	Year	Study Population	Findings
miR-135b	Olasz et al.	2015	11 SCCs from OTRs, 32 SCCs from ICPs, and 15 normal skin samples	Examined 88 cancer-related miRNA and found that miR-135b was the most upregulated in OTRs (21.5-fold in OTRs and 13.3-fold in ICPs; $P = 0.0001$). Upregulation resulted in concomitant decreased expression of LZTS1, as well as increased tumor growth, motility, and invasiveness.
MMP-1	Kuivanen et al.	2009	SCC from 20 ISPs and 20 ICPs	No difference in MMP-1 expression ($P > 0.05$).
MMP-2	Chebassier et al.	2002	SCC from 30 RTRs and 30 ICPs (15 in situ and 15 invasive in each)	Overexpression of MMP-2 was identified in the epidermis surrounding tumor cells in RTRs compared with ICPs with invasive SCC (10 of 15 vs. 6 of 15).
MMP-7	Kuivanen et al.	2009	SCC from 20 ISPs and 20 ICPs	No difference in MMP-7 expression ($P > 0.05$).
MMP-8	Kuivanen et al.	2009	SCC from 20 ISPs and 20 ICPs	No difference in MMP-8 expression ($P > 0.05$).
MMP-9	Kuivanen et al.	2009	SCC from 20 ISPs and 20 ICPs	MMP-9 expression was less abundant in stromal macrophages surrounding SCCs of ISPs ($P = 0.02$).
	Chebassier et al.	2002	SCC from 30 RTRs and 30 ICPs (15 in situ and 15 invasive in each)	Overexpression of MMP-9 was identified in the epidermis surrounding tumor cells in RTRs compared with ICPs with invasive SCC (7 of 15 vs. 4 of 15).
MMP-10	Boyd et al.	2009	SCC from 25 RTRs and 25 ICPs	Stromal expression of MMP-10 occurred in 12% of RTR tumors and 40% of ICP tumors ($P = 0.009$).
MMP-12	Boyd et al.	2009	SCC from 25 RTRs and 25 ICPs	No difference in MMP-12 expression ($P > 0.05$).
MMP-13	Kuivanen et al.	2009	SCC from 20 ISP and 20 ICP	No difference in MMP-13 expression ($P > 0.05$).
MMP-21	Boyd et al.	2009	SCC from 25 RTRs and 25 ICPs	No difference in MMP-21 expression ($P > 0.05$).
MMP-26	Kuivanen et al.	2009	SCC from 20 ISPs and 20 ICPs	MMP-26 expression was significantly more intense in tumor cells of ISPs than in tumor cells of ICPs ($P = 0.01$).
OX40	Feldmeyer et al.	2016	SCC from 10 OTRs and 7 ICPs	A 5.3-fold higher expression of OX40 in OTRs than in ICPs ($P = 0.03$).
p-mTOR (Ser2448)	Gutiérrez-Dalmau et al.	2010	SCC from 37 RTRs and 51 ICPs	p-mTOR was reduced in RTRs when compared with ICPs (28.3 ± 18.7 vs. 55.0 ± 22.1, respectively; $P < 0.001$).
p-p70S6K (Thr421Ser424)	Gutiérrez-Dalmau et al.	2010	SCC from 37 RTRs and 51 ICPs	p-p70S6K was reduced in RTRs when compared with ICPs (30.0 ± 23.0 vs. 45.4 ± 22.5, respectively; $P = 0.026$).
p-Smad1	Harradine et al.	2009	200 SCC lesions from 87 OTRs and 184 lesions from 184 ICPs	No difference in p-Smad1 expression ($P > 0.05$).
p-Smad2	Harradine et al.	2009	200 SCC lesions from 87 OTRs and 184 lesions from 184 ICPs	Increased p-Smad2 staining intensity in OTRs compared with ICPs ($P < 0.001$).
p-Smad5	Harradine et al.	2009	200 SCC lesions from 87 OTRs and 184 lesions from 184 ICPs	No difference in p-Smad5 expression ($P > 0.05$).
p-Smad8	Harradine et al.	2009	200 SCC lesions from 87 OTRs and 184 lesions from 184 ICPs	No difference in p-Smad8 expression ($P > 0.05$).
p14	Küsters-Vandevelde et al.	2009	SCC from 18 RTRs and 16 ICPs	p14 expression was independent of immune status (ICP 8 of 16 [50%] vs. RTR 9 of 18 [50%]; $P > 0.05$).
p16	Küsters-Vandevelde et al.	2009	SCC from 18 RTRs and 16 ICPs	p16 expression was independent of immune status (ICP 9 of 16 [56%] vs. RTR 12 of 18 [67%]; $P > 0.05$).
p53	Gutiérrez-Dalmau et al.	2010	SCC from 37 RTRs and 51 ICPs	p53 staining intensity was greater in SCC from RTRs than in SCC from ICPs (42.1 ± 28.4 vs. 21.8 ± 28.5, respectively; $P = 0.007$).
	Küsters-Vandevelde et al.	2009	SCC from 18 RTRs and 16 ICPs	p53 expression was independent of immune status (ICP 16 of 16 [100%] vs. RTR 12 of 18 [67%]; $P > 0.05$).
	de Graaf et al.	2008	SCC from 19 RTRs and 13 ICPs	p53 patches were more prevalent in RTRs than in ICPs in normal skin adjacent to SCC ($P = 0.02$).
	Blokx et al.	2003	SCC from 44 RTRs and 42 ICPs	SCC tumors in RTRs were more likely to be p53-negative than that in ICPs (30% vs. 0%, respectively; $P = 0.02$).
	Seçkin et al.	2002	SCC from 10 RTRs and 14 ICPs	No difference in p53 expression ($P > 0.05$).
PD-L1	Varki et al.	2018	SCC from 42 ICPs and 24 ISPs (13 OTRs, 8 HIV, and 3 others)	No difference in PD-L1 expression ($P = 0.05$).
Psoriasin (S100A7)	Muehleisen et al.	2012	SCC from 11 OTRs and 17 ICPs	Psoriasin levels in the tumor center of OTRs were significantly lower than in ICPs (71.9 ± 49.1 vs. 1334 ± 690; $P < 0.01$).
RAGE	Iotzova-Weiss et al.	2015	SCC from 13 OTRs and 19 ICPs	No significant difference in RAGE expression ($P > 0.05$).
S100A8	Iotzova-Weiss et al.	2015	SCC from 13 OTRs and 19 ICPs	S100A8 expression was significantly higher in OTRs with invasive SCC than in ICPs with invasive SCC ($P < 0.01$).
S100A9		2015	SCC from 13 OTRs and 19 ICPs	

(*continued*)

Table 1. Continued

Proteins/Genes of interest	Author	Year	Study Population	Findings
	Iotzova-Weiss et al.			S100A9 expression was significantly higher in OTRs with invasive SCC than in ICPs with invasive SCC ($P < 0.05$).
T-BET	Kosmidis et al.	2010	SCC from 42 OTRs and 43 ICPs	*TBET* mRNA expression was decreased in OTRs compared with ICPs ($P = 0.0056$; OTR mean 4.17×10^{-5}, SD $\pm 5.05 \times 10^{-5}$, interquartile range 1.28×10^{-5} to 5.68×10^{-5}; ICP mean 1.65×10^{-4}, SD $\pm 1.66 \times 10^{-4}$, interquartile range 4.4×10^{-5} to 2.38×10^{-4}).
TGFβ	Kosmidis et al.	2010	SCC from 42 OTRs and 43 ICPs	*TGFB* mRNA was decreased in OTRs compared with ICPs ($P = 0.036$; OTR mean 8.93×10^{-3}, SD $\pm 4.8 \times 10^{-3}$, interquartile range 3.96×10^{-3} to 13.2×10^{-3}; ICP mean 13.91×10^{-3}, SD $\pm 5.9 \times 10^{-3}$, interquartile range 9.97×10^{-3} to 17.53×10^{-3}).
	Harradine et al.	2009	200 SCC lesions from 87 OTRs and 184 lesions from 184 ICPs	No difference in TGFβ expression ($P > 0.05$).
	Gutiérrez-Dalmau et al.	2010	SCC from 37 RTRs and 51 ICPs	TGFβ staining intensity was greater in SCC from RTRs than in SCC from ICPs (2.08 ± 0.97 vs. 1.63 ± 0.77, respectively; $P = 0.049$).
TIMP-1	Kuivanen et al.	2009	SCC from 20 ISPs and 20 ICPs	No difference in TIMP-1 expression ($P > 0.05$).
TIMP-3	Kuivanen et al.	2009	SCC from 20 ISPs and 20 ICPs	No difference in TIMP-3 expression ($P > 0.05$).
TβRII	Harradine et al.	2009	200 SCC lesions from 87 OTRs and 184 lesions from 184 ICPs	TβRII showed a slight but significant reduction in expression in SCCs from OTRs than in SCCs from ICPs ($P = 0.03$).
XPC	de Feraudy et al.	2010	SCC from 34 OTRs and 35 ICPs	XPC expression was lost in 26–49% of invasive SCC from ICPs and 50–59% from OTRs ($P = 0.08$).

Abbreviations: BD, beta defensin; cSCC, cutaneous squamous cell carcinoma; hBD, human β-defensin; hTERT, human telomerase reverse transcriptase; ICP, immunocompetent patient; IHC, immunohistochemistry; ISP, immunosuppressed patient; miRNA, microRNA; MMP, matrix metalloproteinase; OTR, organ-transplant recipient; p-70S6K, phosphorylated 70S6K; p-mTOR, phosphorylated mTOR; p-Smad, phosphorylated Smad; RTR, renal transplant recipient; SCC, squamous cell carcinoma; Ser, serine; Th1, T helper type 1; Thr, threonine.
A summary of gene expression differences in cSCC in ISPs compared with ICPs.

MTOR and its downstream target P70-S6 kinase 1 were lower in RTRs than in ICPs ($P < 0.001$ and $P = 0.026$, respectively) (Gutiérrez-Dalmau et al., 2010). *OX40*, which activates NF-κ to suppress apoptosis, was expressed more highly in tumors from OTRs ($P = 0.03$) (Feldmeyer et al., 2016) Expression of the anti-apoptotic protein Bcl-xL was decreased in RTRs compared with that in ICPs, whereas no difference was observed in the expression of the pro-apoptotic proteins Bax and Bcl-2 ($P = 0.042$, $P > 0.05$, and $P > 0.05$, respectively) (Burke et al., 2015; Seçkin et al., 2002). Expression of the DNA damage repair protein XPC was lost at higher rates in tumors from OTRs than in ICPs ($P = 0.08$) (de Feraudy et al., 2010). Of the examined microRNAs (miRNAs) in OTRs, miR-135b was the most upregulated, by 21.5-fold in OTRs and 13.3-fold in ICPs ($P = 0.0001$) (Olasz et al., 2015).

Reports on the comparative expression of matrix metalloproteinases (MMPs) (Zou et al., 2021), a family of proteins reported to be associated with tumor progression, showed mixed results. Chebassier et al. (2002) found higher levels of MMP-2 and MMP-9 in the peritumoral skin of RTRs than that in the peritumoral skin of ICPs (10 of 15 vs. 6 of 15 and 7 of 15 vs. 4 of 15, respectively), and Kuivanen et al. (2009) found that intratumoral expression of *MMP26* was increased among tumors from ISPs compared with that among tumors from ICPs ($P = 0.01$). Conversely, the same study showed that MMP-9 was less abundant in tumor-associated macrophages of ISPs ($P = 0.02$) (Kuivanen et al., 2009), and Boyd et al. (2009) showed that squamous cell carcinoma (SCC) from RTRs was less likely to express *MMP10* than tumors from ICPs ($P = 0.009$).

Several studies examined the expression of p53, and two groups found increased staining intensity of p53 within OTRs ($P = 0.007$ and $P = 0.02$, respectively) (de Graaf et al., 2008; Gutiérrez-Dalmau et al., 2010). On the contrary, Küsters-Vandevelde et al. (2009) found that levels of both mRNA and protein for the tumor suppressor genes *P14*, *P16*, and *P53* were independent of immune status ($P > 0.05$). Another study by Seçkin et al. (2002) also found no difference in p53 expression ($P > 0.05$). In contrast, Blokx et al. (2003) showed a higher prevalence of p53-negative tumors in cSCC from RTRs than in ICP tumors (30% vs. 0%, respectively; $P = 0.02$). Together, the results from different studies are entirely inconsistent.

Data on *TGFB* expression was also equivocal. TGFβ signaling is often dysregulated in human cancer, with tumor suppressive function in premalignant and early-stage lesions and oncogenic effects in more advanced lesions (Zhang et al., 2021). One study found *TGFB* mRNA levels to be decreased in tumors from OTRs ($P = 0.036$) (Kosmidis et al., 2010), whereas another showed TGFβ staining to be more intense among the RTR population ($P = 0.049$) (Gutiérrez-Dalmau et al., 2010). A third study by Harradine et al. (2009) found no difference in *TGFB* expression ($P > 0.05$) but did show that tumors from ISPs had increased staining for phosphorylated Smad (p-Smad) 2 ($P < 0.001$), which is downstream of TGFβ to promote growth and differentiation. Levels of p-Smad1, p-Smad5, and p-Smad8 were not different between the two groups ($P > 0.05$) (Harradine et al., 2009). The same study also showed decreased expression of the type II TGFβ receptor in cSCCs from OTRs ($P = 0.03$) (Harradine et al., 2009).

Table 2. Gene Expression Associated with cSCC in ISPs

Proteins/ Genes of interest	Author	Year	Study Population	Findings
CD57	Bottomley et al.	2015	110 RTRs (57 with SCC and 53 without)	RTRs exhibiting a population of >50% of CD8+ T cells expressing CD57 were at significantly greater risk of developing a future SCC (HR = 5, 95% CI = 1.11−22.3, $P = 0.04$).
CGRP	Frauenfelder et al.	2017	SCC from 34 OTRs (18 pain-associated tumors, 16 without)	No difference in CGRP expression levels in SCC with pain compared with SCC without pain in OTRs.
FOXP3	Sherston et al.	2014	57 RTRs with SCC and 49 RTRs without SCC	Proportion of CD4+ FOXP3+ cells was higher in RTRs with SCC than in RTRs without SCC ($P = 0.017$).
HLA-A11	Bouwes Bavinck et al.	1997	1,098 RTRs of whom 271 developed SCC	Expression of HLA antigen HLA-A11 was associated with increased risk of skin cancer in RTRs (RR = 1.7, 95% CI = 1.1−2.4, $P = 0.009$).
HLA-DRB1*13	Kim et al.	2020	46 RTRs who developed cSCC after transplant	HLA-DRB1*13 was associated with SCC risk in RTRs after transplant (HR = 2.24, 95% CI = 1.12−4.49, $P = 0.023$).
HLA-G	Aractingi et al.	2003	37 SCC from RTRs and 24 benign lesions from RTRs	HLA-G expression was higher in SCC ($P < 0.02$, Fischer's exact test) and in Bowen's disease ($P < 0.004$, Fischer's exact test) than in benign lesions. The level of positivity was not different when comparing SCC and Bowen's disease ($P = 0.25$).
IL-1β	Frauenfelder et al.	2017	SCC from 34 OTRs (18 pain-associated tumors and 16 without)	No difference in IL-1β expression levels in SCC with pain compared with SCC without pain in OTRs.
mir-1246	Geusau et al.	2020	Eight OTRs with cSCC and eight OTRs without cSCC	mir-1246 was significantly upregulated in both tumor tissue and serum in OTRs with cSCC compared to those without (p = 0.013).
mir-1290	Geusau et al.	2020	8 OTRs with cSCC and 8 OTRs without cSCC	mir-1290 was significantly upregulated in both tumor tissue and serum in OTRs with cSCC compared with those without ($P = 0.037$).
NGF	Frauenfelder et al.	2017	SCC from 34 OTRs (18 pain-associated tumors, 16 without)	No difference in NGF expression levels in SCC with pain compared with SCC without pain in OTRs.
p53	Maurer et al.	1997	SCC and normal skin from 33 patients with HIV excised from both UV-exposed and UV-protected areas	92% (22 of 24) of SCC specimens and 95% (17 of 20) of tissue specimens adjacent to SCCs stained for p53, whereas control specimens from UV-protected skin did not stain for p53.
PGE_2	Frauenfelder et al.	2017	SCC from 34 OTRs (18 pain-associated tumors and 16 without)	SCC with pain is associated with increased levels of PGE_2 (OR = 1.9, 95% CI = 1.1−3.4, $P = 0.002$), adjusted for age and sex.
POMC	Frauenfelder et al.	2017	SCC from 34 OTRs (18 pain-associated tumors, 16 without)	SCC with pain was associated with increased levels of (POMC) compared with SCC without pain (OR = 1.5, 95% CI = 0.99−2.0, $P = 0.05$), adjusted for age and sex.
TNF-α	Frauenfelder et al.	2017	SCC from 34 OTRs (18 pain-associated tumors and 16 without)	SCC with pain was associated with increased levels of TNF-α compared with SCC without pain (adjusted OR = 1.4, 95% CI = 0.99−2.0, $P = 0.05$).

Abbreviations: CI, confidence interval; cSCC, cutaneous squamous cell carcinoma; HR, hazard ratio; ICP, immunocompetent patient; ISP, immunosuppressed patient; NGF, nerve GF; OTR, organ-transplant recipient; PGE_2, prostaglandin E2; POMC, pro-opiomelanocortin; RR, relative risk; RTR, renal transplant recipient; SCC, squamous cell carcinoma.

A summary of gene expression associated with cSCC in ISPs.

The expression of the S100 family of calcium sensing proteins was also probed. One study found that S100A7 levels were lower in cSCC from OTRs than from ICPs ($P < 0.01$) (Muehleisen et al., 2012). Another study looking at the expression of *S100A8* and *S100A9*, showed higher expression in invasive cSCC from OTRs than from ICPs ($P < 0.01$ and $P < 0.05$, respectively) (Iotzova-Weiss et al., 2015). In other tumor types, S100A7 has been associated with decreased proliferation and metastases, whereas S100A8 and S100A9 are associated with increased growth and immune evasion (Bresnick et al., 2015). Human β-defensin (hBD) levels were measured by Muehleisen et al. (2012), showing that hBD-1 and hBD-2 expression was increased in tumors from ICPs compared with normal skin ($P < 0.05$ and $P < 0.01$, respectively). However, this overexpression was only seen with hBD-2 among ISPs and not hBD-1 ($P < 0.02$ and $P > 0.05$, respectively) (Muehleisen et al., 2012). hBDs are important components of the innate immune system in the skin but have controversial roles in oncogenesis.

Some studies focused on the expression of immune cell markers. The expression of T-Bet, which promotes the T helper type 1 phenotype, was decreased in OTRs compared with that in ICPs in one study ($P = 0.0056$) (Kosmidis et al., 2010), and CD4+ cells were decreased in tumors from OTRs ($P < 0.05$) (Zhang et al., 2013). Consistently, the CD8+ T cells were decreased and the expression of FOXP3 was higher, whereas absolute FOXP3 expression was diminished in OTRs compared with that in ICPs ($P = 0.013$, $P < 0.05$, and $P = 0.45$, respectively) (Carroll et al., 2010; Frazzette et al., 2020; Kosmidis et al., 2010; Zhang et al., 2013). B7-H3, a known oncogene and immune checkpoint molecule was shown to be widely expressed in tumors from ICPs ($P = 0.025$) (Varki et al., 2018), and IFN-γ, a key regulator of innate and adaptive immunity, was expressed more highly among tumors from ICPs ($P = 0.02$) (Kosmidis et al., 2010). Other key immune responses, IL-17A and IL-22R, had lower expression in tumors from ISPs ($P = 0.016$ and $P < 0.05$, respectively) (Table 1) (Kosmidis et al., 2010; Zhang et al., 2013).

Table 3. Gene Polymorphisms and Haplotypes

Gene of interest	Author	Year	Study population	Polymorphism	Findings
3′UTR of *PTGS2*	Gomez-Lira et al.	2011	138 OTRs with SCC and 124 OTRs without SCC	+8473T>C +8293G>C +10259T>G +10267G>A +10335G>A	No allele frequency differences were observed between cases and controls for any of the identified polymorphisms, suggesting that polymorphisms in the 3′UTR of the *PTGS2* gene are rare and unlikely to represent risk factor for NMSC after transplantation.
	Lira et al.	2007	107 OTRs with SCC and 133 OTRs without SCC	+8473T>C	Variant +8473T>C, located in the 3′UTR region of the gene, showed no association with NMSC risk after transplantation.
9p21-22 (*p16INK4* and *p14ARF*)	Mühleisen et al.	2012	42 OTRs with SCC and 43 ICPs with SCC	D9S162	Allelic balance at D9S162 was reduced for SCC in OTRs compared with SCC in ICPs ($P = 0.04$).
ASIP haplotype	Andresen et al.	2013	80 RTRs with SCC and 137 RTRs without SCC	rs4911414 rs1015362	A tendency toward an increased SCC risk was observed for a specific ASIP haplotype (OR = 1.87, 95% CI = 0.91−3.83), but the association was not statistically significant.
CACNA1D	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs3774611 A allele rs893365 T allele	Two SNVs at the *CACNA1D* gene had a significant association with the development of NMSC in OTRs (OR = 2.67, 95% CI = 1.73−4.10, $P = 8.01 \times 10^{-6}$; OR = 2.67, 95% CI = 1.73−4.10, $P = 8.01 \times 10^{-6}$, respectively).
CDK10	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs258322 A allele	An SNV at the *CDK10* gene had a significant association with the development of NMSC in OTRs (OR = 2.07, 95% CI = 1.14−3.74, $P = 0.02$).
COX2 gene promoter	Aubin et al.	2010	11 OTRs with SCC and 592 OTRs without SCC	−76hl5	Statistical analysis showed no difference between the genotypic distribution of RTRs presenting with skin cancer and those without a history of skin cancer.
CSMD1	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs13270945 A allele rs9644363 G allele rs34851282 T allele	Three SNVs at the *CSMD1* gene had a significant association with the development of NMSC in OTRs (OR = 3.14, 95% CI = 1.90−5.20, $P = 8.78 \times 10^{-6}$; OR = 3.14, 95% CI = 1.90−5.20, $P = 8.78 \times 10^{-6}$; OR = 3.15, 95% CI = 1.90−5.25, $P = 9.64 \times 10^{-6}$, respectively).
DLEU7	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs1239947 T allele	An SNV at the *DLEU7* gene had a significant association with NMSC development in OTRs (OR = 1.58, 95% CI = 1.02−2.47, $P = 0.04$).
EGF	Tartaglia et al.	2007	95 OTRs with SCC and 106 OTRs without SCC	rs4444903 G allele	Genotype and the allele frequencies were not significantly different between the control group and the transplanted patients with and without skin tumors.
EXOC2	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs12210050 T allele	An SNV at the *EXOC2* gene had a significant association with the development of NMSC in OTRs (OR = 1.95, 95% CI = 1.16−3.20, $P = 0.01$).
GSTM1	Fryer et al.	2005	361 RTRs with SCC and without	*GSTM1* AB allele	RTRs carrying the *GSTM1* AB allele had a decreased risk of developing NMSC when compared with other alleles at the same locus (RR = 0.23, 95% CI = 0.05−0.99, $P = 0.049$).
	Ramsay et al.	2001	183 RTRs with SCC and without	*GSTM1* null	A significant association was found in RTRs carrying *the GSTM1* null allele and the development of NMSC (OR = 8.4, $P = 0.012$).
	Marshall et al.	2000	222 RTRs with SCC and without	*GSTM1* A/B/AB/null	No significant association of the *GSTM1* genotype between groups.
GSTM1 and *CYP1A1* haplotype	Lira et al.	2006	107 OTRs with SCC and 132 OTRs without SCC	*GSTM1* null & *CYP1A1* Val^{462}	A haplotype including the *GSTM1* and *CYP1A1* genes was identified with a significant association with the development of NMSC in OTRs (OR = 6.5, 95% CI = 1.4−34.4, $P = 0.01$).
GSTM3	Fryer et al.	2005	361 RTRs with SCC and without	*GSTM3* AA allele	RTRs with the *GSTM3* AA allele had a reduced risk of SCC (RR = 0.50, 95% CI = 0.28−0.87, $P = 0.015$).
	Ramsay et al.	2001	183 RTRs with SCC and without	*GSTM3* AA/AB/BB	No significant association of the *GSTM3* genotype between groups.
GSTP1	Lira et al.	2006	107 OTRs with SCC and 132 OTRs without SCC	Val^{105}	OTRs with the Val^{105} polymorphism in the *GSTP1* gene showed a significantly decreased risk of developing NMSC (OR = 0.1, 95% CI = 0.0−0.7, $P = 0.012$).
	Ramsay et al.	2001	183 RTRs with SCC and without	*GSTP1**Ile/Ile	A significant association was found in RTRs carrying the *GSTP1**Ile/Ile allele and the development of NMSC (OR = 7.6, $P = 0.002$).
	Marshall et al.	2000	222 RTRs with SCC and without	*GSTP1**C allele	*GSTP1**C allele was associated with the development of SCC ($P = 0.01$).

(*continued*)

Table 3. Continued

Gene of interest	Author	Year	Study population	Polymorphism	Findings
GSTP1A haplotype	Lira et al.	2006	107 OTRs with SCC and 132 OTRs without SCC	Ile^{105} & Ala^{114}	A *GSTP1A* haplotype was identified with a significant association with the development of NMSC in OTRs (OR = 1.7, 95% CI = 1.1−2.5, $P = 0.017$).
GSTT1	Ramsay et al.	2001	183 RTRs with SCC and without	*GSTT1* null/A	No significant association of the *GSTT1* genotype between groups.
	Marshall et al.	2000	222 RTRs with SCC and without	*GSTT1* null	No significant association of the *GSTT1* genotype between groups.
HERC2	Wei et al.	2017	386 OTRs with SCC and without	rs916977 brown eye allele compared with blue eye allele and rs12913832 brown eye allele compared with blue eye allele	OTRs homozygous for brown eye alleles rs916977 (GG) and rs12913832 (AA) had significant delays of time to first cSCC after transplant compared with individuals homozygous for blue eye alleles (HR = 0.34, $P < 0.001$; HR = 0.54, $P = 0.012$, respectively).
HLADQ1	Asgari et al.	2016	61,457 SCC (ICPs and ISPs)	rs4455710 allele	An intronic polymorphism at the *HLA* locus at 6p21 in the *HLADQA1* gene was strongly associated with increased risk of SCC in ISPs (HR = 1.17, $P = 1.86 \times 10^{-8}$).
Hp	Speeckaert et al.	2012	300 RTRs	*Hp* polymorphism	Significant association of the *Hp 1-1* phenotype with a higher risk of SCC/Bowen's disease ($P = 0.035$) and multiple primary SCCs ($P = 0.002$). No significant difference between the *Hp* phenotypes was found in the first 10 years after transplantation. However, after a follow-up of >10 y, significant association between *Hp 1-1* phenotype and the occurrence of Bowen's disease and SCC ($P = 0.002$ and $P = 0.001$, respectively).
IL10 gene promoter haplotypes	Alamartine et al.	2003	40 RTRs with SCC, 70 RTRs without SCC, and 70 ICPs	$A\text{-}_{1082}\text{-}T\text{-}_{819}\text{-}A\text{-}_{592}$ (IL-10 low) $G\text{-}_{1082}\text{-}C\text{-}_{819}\text{-}C\text{-}_{592}$ (IL-10 high)	Two haplotypes in the *IL10* gene promoter were identified in RTRs which resulted in different levels of IL-10 expression. IL-10 low had a protective effect on the development of NMSC (OR = 0.16, 95% CI = 0.06−0.42, $P = 0.05$), whereas IL-10 high had a significant association with the development of NMSC (OR = 2.58, 95% CI = 1.05−7.04, $P = 0.05$).
Intergenic (CHR 5)	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs4460176 T allele	An SNV, rs4460276 T allele, at an intergenic location on CHR 5 had a significant association with NMSC development in OTRs (OR = 1.62, 95% CI = 1.08−2.43, $P = 0.02$).
Intergenic (CHR 6)	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	Intergenic rs1540771 T allele	An SNV, rs1540771, on CHR 6 had a significant association with the development of NMSC in OTRs (OR = 1.89, 95% CI = 1.26−2.82, $P = 0.002$).
Intergenic (CHR 11)	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs11820512 A allele	An SNV, rs11820512 A allele, at an intergenic location on CHR 11 had a significant association with the development of NMSC in OTRs (OR = 6.66, 95% CI = 2.91−15.22, $P = 7.10 \times 10^{-6}$).
Intergenic (CHR 18)	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs4799088 A allele	An SNV, rs4799088 A allele, at an intergenic location on CHR 18 had a significant association with NMSC development in OTRs (OR = 1.66, 95% CI = 1.03−2.67, $P = 0.04$).
IRF4	Asgari et al.	2017	388 OTRs with SCC and without	rs12203592 T allele	In univariate analysis, the *IRF4* rs12203592 T allele was associated with a significantly increased hazard for time to first cSCC (HR = 1.36, $P = 0.02$); this association was maintained when adjusted for sex, age, organ transplanted, and Fitzpatrick skin type (HR = 1.34, $P = 0.04$).
	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs12203592 T allele	An SNV at the *IRF4* gene had a significant association with the development of NMSC in OTRs (OR = 2.08, 95% CI = 1.23−3.53, $P = 0.007$).
LINC00882	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs6791146 C allele rs68025055 rs13061320 C allele rs34796852 T allele	Four SNVs at the *LINC00882* gene had a significant association with NMSC development in OTRs (OR = 3.79, 95% CI = 2.17−6.60, $P = 2.76 \times 10^{-6}$; OR = 3.71, 95% CI = 2.13−6.48, $P = 4.01 \times 10^{-6}$; OR = 3.71, 95% CI = 2.12−6.47, $P = 4.12 \times 10^{-6}$; OR = 3.71, 95% CI = 2.12−6.47, $P = 4.12 \times 10^{-6}$, respectively).
MC1R	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs4785763 A allele	An SNV at the *MC1R* gene had a significant association with the development of NMSC in OTRs (OR = 1.62, 95% CI = 1.07−2.45, $P = 0.02$).

(*continued*)

Table 3. Continued

Gene of interest	Author	Year	Study population	Polymorphism	Findings
MC1R haplotype	Andresen et al.	2013	80 RTRs with SCC and 137 RTRs without SCC	rs1805007 (red hair) Any two of the following in combination: rs1805006 rs1805007 rs1805008 rs1805009 rs1805005 rs2228479 rs885479	Significant associations with SCC risk in RTRs were indicated in carriers of the red hair color associated *MC1R* variant p.Arg151Cys (OR = 1.99, 95% CI = 1.05−3.75), and in carriers of two of any of the MC1R variants included to the left (OR = 2.36, 95% CI = 1.08−5.15).
MTHFR	Laing et al.	2007	117 RTRs with SCC and 250 RTRs without SCC	rs1801133 T allele	Individuals carrying the *MTHFR* 677T allele had a marked increase in risk of SCC (adjusted OR = 2.54, P = 0.002), after adjustment for age, gender, skin type, sun exposure score, and immunosuppression duration.
MYPN	Sanders et al.	2015	88 OTRs with SCC and 300 OTRs without SCC	rs6480314 G allele	An SNV at the *MYPN* gene had a significant association with NMSC development in OTRs (OR = 1.66, 95% CI = 1.02−2.71, P = 0.04).
OCA2	Wei et al.	2017	386 OTRs with SCC and without	rs916977 brown eye allele compared with blue eye allele and rs12913832 brown eye allele compared with blue eye allele	OTRs homozygous for the brown eye alleles of rs916977 (GG) and rs12913832 (AA) had significant delays of time to first cSCC after transplant than those individuals homozygous for the blue eye alleles (HR = 0.34, $P < 0.001$; HR = 0.54, P = 0.012, respectively).
Promoter of *PTGS2*	Lira et al.	2007	107 OTRs with SCC and 133 OTRs without SCC	−765G>C, −1195A>G	Variant −1195G was over-represented in patients with SCC undergoing transplantation after 50 y of age, but this difference did not reach significance (P = 0.09).
PTGS2 Haplotype	Lira et al.	2007	107 OTRs with SCC and 133 OTRs without SCC	G_{-1195}-G_{-765}-T_{-8473}	A significant association between a *PTGS2* haplotype and development of NMSC in OTRs was identified (OR = 4.77, 95% CI = 1.47−16.41, P = 0.01).
RNF5P1	Sanders et al.	2015	88 OTR with SCC and 300 OTR without SCC	rs7832232 A allele	An SNV at the *RNF5P1* gene had a significant association with NMSC development in OTRs (OR = 1.50, 95% CI = 1.02−2.21, P = 0.04).
SLC45A2	Asgari et al.	2017	388 OTRs with SCC and without	rs16891982 C allele	The *SLC45A2* rs16891982 C allele was associated with a decreased hazard for cSCC in univariate analysis (HR = 0.58, P = 0.04), and the effect was similar but not significant in the multivariate model (HR = 0.74, P = 0.06).
TLR4	Laing et al.	2009	168 RTRs with SCC and 286 RTRs without SCC	rs4986790 rs179008 rs3764880	No significant association of the *TLR4* genotype between groups.
TLR7	Laing et al.	2009	168 RTRs with SCC and 286 RTRs without SCC	rs4986790 rs179008 rs3764880	No significant association of the *TLR7* genotype between groups.
TLR8	Laing et al.	2009	168 RTRs with SCC and 286 RTRs without SCC	rs4986790 rs179008 rs3764880	No significant association of the *TLR8* genotype between groups.
TMC6/EVER1	Burger et al.	2015	16 RTRs with SCC and 25 RTRs without SCC	26 SNVs within both *TMC* and *EVER* genes	No significant association was found between any SNV genotype within *TMC6/EVER1* and risk of SCC development in RTRs.
TMC8/EVER2	Burger et al.	2015	16 RTRs with SCC and 25 RTRs without SCC	26 SNVs within both *TMC* and *EVER* genes	No significant association was found between any SNV genotype within *TMC8/EVER2* and risk of SCC development in RTRs.
TP53	Cairey-Remonnay et al.	2002	53 RTRs with SCC, 50 RTRs with benign lesions, 41 ICPs with SCC, and 29 blood samples from ICPs without SCC	Codon 72 of exon 4	Rate of arginine homozygosity in SCC from RTRs was significantly higher (83%) than in ICPs with or without SCC (60% and 59%, respectively).
TYR	Andresen et al.	2013	80 RTRs with SCC and 137 RTRs without SCC	rs1126809	No significant association of the *TYR* genotype between groups.
TYRP1	Andresen et al.	2013	80 RTRs with SCC and 137 RTRs without SCC	rs1408799	No significant association of the *TYRP1* genotype between groups.

(*continued*)

Table 3. Continued

Gene of interest	Author	Year	Study population	Polymorphism	Findings
Upstream of *RP1163E5.6*, *FBXO25*, and *OR4F2*	Kuzmanov et al.	2019	61 OTRs with cSCC and 908 OTRs without cSCC	rs34567942	GWAS identified one SNV, rs34567942, to be significantly associated with cSCC in OTRs at the *P*-value threshold of 5×10^{-8}.

Abbreviations: CHR, chromosome; CI, confidence interval; cSCC, cutaneous squamous cell carcinoma; HR, hazard ratio; ICP, immunocompetent patient; ISP, immunosuppressed patient; NMSC, nonmelanoma skin cancer; OTR, organ-transplant recipient; RR, relative risk; RTR, renal transplant recipient; SCC, squamous cell carcinoma; UTR, untranslated region.

A summary of polymorphisms identified in the ISP population with an associated risk of SCC.

The other nine studies (12% of all articles) assessed differences in protein and mRNA expression in ISPs with and without cSCC, with a total of 16 unique proteins/genes of interest being identified (Table 2). Some studies reported the circulating host immune phenotype. One study found that the population of circulating regulatory T cells was higher in RTRs with SCC than in those without ($P = 0.017$) (Sherston et al., 2014). Patients with higher levels of CD8+ T cells expressing the senescence marker CD57 were at higher risk for developing cSCC (hazard ratio [HR] = 5, 95% confidence interval [CI] = 1.11−22.3, $P = 0.04$) (Bottomley et al., 2015). Expression of HLA haplotypes HLA-A11 (relative risk [RR] = 1.7, 95% CI = 1.1−2.4, $P = 0.009$) and HLA-DRB1*13 (HR = 2.24, 95% CI = 1.12−4.49, $P = 0.023$) was associated with increased SCC risk in RTRs (Bouwes Bavinck et al., 1997; Kim et al., 2020). HLA-G, thought to act as an immune checkpoint (Zaborek-Łyczba et al., 2021), was expressed more highly in malignant lesions from RTRs than in benign lesions in the same population ($P < 0.02$) (Aractingi et al., 2003).

Geusau et al. (2020) examined the tissue and circulating expression of miRNAs in patients and found that mir-1246 and mir-1290 were associated with cSCC in OTRs ($P = 0.013$ and $P = 0.037$, respectively). In a study on OTRs with SCC, expression of PGE2 (OR = 1.9, 95% CI = 1.1−3.4, $P = 0.002$), POMC (OR = 1.5, 95% CI = 0.99−2.0, $P = 0.05$), and TNF-α (adjusted OR = 1.4, 95% CI = 0.99−2.0, $P = 0.05$) was associated with painful lesions (Table 2) (Frauenfelder et al., 2017).

Gene polymorphisms and haplotypes

Of the reviewed articles, 21 studies (27% of all articles) discussed gene polymorphisms (germline mutations) or associated haplotypes and the associated risk of cSCC in ISPs and OTRs (Table 3). A total of 44 specific genes of interest and associated polymorphisms or haplotypes were delineated. One study evaluated chromosomal inactivation at 9p21-22 and found broadly reduced allelic balance at 9p21-22 in both OTRs and ICPs; however, a unique microsatellite location, D9S162, was identified in OTRs that showed reduced allelic balance when compared with ICPs ($P = 0.04$) (Mühleisen et al., 2012). Alleles identified with the greatest associated risk included genes coding for the glutathione S-transferase supergene family including *GSTM1 null* (OR = 8.4, $P = 0.012$) and *GSTP1*Ile/Ile* (OR = 7.6, $P = 0.002$) (Ramsay et al., 2001). The nonfunctional intergenic variant polymorphism rs11820512 A allele on chromosome 11 (OR = 6.66, 95% CI = 2.91−15.22, $P = 7.10 \times 10^{-6}$) was also highly associated with cSCC development, as well as a group of polymorphisms in the *LINC00882* gene (OR = 3.79, 95% CI = 2.17−6.60, $P = 2.76 \times 10^{-6}$; OR = 3.71, 95% CI = 2.13−6.48, $P = 4.01 \times 10^{-6}$; OR = 3.71, 95% CI = 2.12−6.47, $P = 4.12 \times 10^{-6}$; and OR = 3.71, 95% CI = 2.12−6.47, $P = 4.12 \times 10^{-6}$) (Sanders et al., 2015). Haplotypes with the greatest risk for cSCC included the combination of *GSTM1 null* and the cytochrome P450 allele *CYP1A1* Val^{462} (OR = 6.5, 95% CI = 1.4−34.4, $P = 0.01$) (Lira et al., 2006), as well as the COX-2 encoding gene *PTGS2* haplotype G_{-1195}-G_{-765}-T_{-8473} (OR = 4.77, 95% CI = 1.47−16.41, $P = 0.01$) (Lira et al., 2007). A protective role was found for several alleles located within genes influencing pigmentation, including the brown eye allele at *OCA1/HERC2* and the C allele for *SLC45A2* (Asgari et al., 2017; Wei et al., 2017). OTRs homozygous for the brown eye alleles rs916977 (GG) and rs12913832 (AA) had significant delays of time to first cSCC post-transplant compared with individuals homozygous for the blue eye alleles (HR = 0.34, $P < 0.001$ and HR = 0.54, $P = 0.012$, respectively) (Wei et al., 2017), whereas the *SLC45A2* rs16891982 C allele was associated with a decreased HR for cSCC in univariate analysis (HR = 0.58, $P = 0.04$) (Asgari et al., 2017). In contrast to the increased risk shown by *GSTM1 null*, a protective effect was found for the *GSTM1 AB allele* (RR = 0.23, 95% CI = 0.05−0.99, $P = 0.049$) (Fryer et al., 2005). Greatly reduced risk of developing cSCC was also found in those ISPs who possessed the A_{-1082}-T_{-819}-A_{-592} (IL-10 low) haplotype (OR = 0.16, 95% CI = 0.06−0.42, $P = 0.05$) (Table 3) (Alamartine et al., 2003).

Drug-induced alterations

This review found 15 articles (19% of all articles) that examined the effects of calcineurin inhibitors (CNIs), such as cyclosporine (CsA) and tacrolimus (Table 4). Treatment with CsA was shown to hasten tumor growth, upregulate IL-22R expression, and cause increased Jak1, signal transducer and activator of transcription (STAT) 1, and STAT3 expression ($P < 0.05$) (Abikhair et al., 2016; Abikhair Burgo et al., 2018). CsA was also shown to enhance production of proinflammatory cytokines by decreasing expression of tristetraprolin ($P < 0.05$) (Wu et al., 2018). In addition, ATF3 was shown to be selectively induced by CNIs and an additive effect was found between CsA and UVA (Dziunycz et al., 2014; Wu et al., 2010). In a mouse model, Goldstein et al. (2015) revealed increased signaling of *Nfatc1* in CsA-

treated mice, with associated increased spontaneous SCC formation (Goldstein et al., 2015). Other murine studies found that tacrolimus-treated mice showed higher numbers of chromosomal aberrations than CsA, sirolimus, and mycophenolate (Dworkin et al., 2009), and that IFN-γ—neutralization in tacrolimus-treated mice abrogated SCC regression, significantly reducing CD8+ T-cell infiltration into SCC, and significantly impairing the secretion of CXCL9, CXCL10, and CCL5 within the tumor microenvironment (Zeng et al., 2021). Other notable findings included reduced expression intensity of Mcl-1 in tacrolimus-treated RTRs compared with sirolimus-treated RTRs and ICPs (Burke et al., 2015), augmented tumor growth in CsA-treated human cells lines through activation of TGFβ-activated kinase 1 (TAK1) (Xu et al., 2011), and greater abundance of MMP-26 expression in cancer cells ($P = 0.04$), and MMP-9 in neutrophils ($P = 0.005$) in SCCs of patients taking CsA (Table 4) (Kuivanen et al., 2009).

Six articles (8% of all articles) discussed genetic alterations owing to AZA (Table 5). AZA was shown to lead to the accumulation of 6-thioguanine (6-TG) in cellular DNA (0.02% substitution of DNA guanine vs. 0% in those not treated with AZA), which has a greater absorption potential for UVA than normal DNA, and thus increased development of cutaneous malignancies (O'Donovan et al., 2005) while also reducing DNA repair activity (Brem et al., 2010). In addition, a previously unknown mutational signature in cSCC of OTRs, termed signature 32, was discovered during whole exome sequencing and mutational signature analysis of OTRs (Inman et al., 2018). Analysis of treatment times revealed a strong positive correlation with the estimated time of AZA exposure and the prevalence of signature 32 (Spearman's rank order correlation r_s (26) = 0.679, $P < 0.0001$) (Inman et al., 2018). In a mouse model, Kalra et al. (2012, 2011) revealed that genetic upregulation of Keap1/Nrf2/ARE reduces incorporation of 6-TG in DNA after treatment with AZA and that a robust systemic induction of the Keap1/Nrf2/ARE pathway protects cells with 6-TG incorporations against oxidative stress caused by UVA radiation (Table 5) (Kalra et al., 2012, 2011).

One article (1% of all articles) described cSCC risk associated with the antifungal medication voriconazole (Table 6). Voriconazole was associated with a 73% increased risk for cSCC development in lung transplant recipients. Allelic variant *17 of *CYP2C19* had a 74% increased hazard for SCC (95% CI = 1.06—2.84, $P = 0.03$) (Table 6) (Williams and Arron, 2016).

Four articles (5% of all articles) reported on gene expression changes associated with mTOR inhibitors (Table 7). Sirolimus was reported to downregulate the ATF3 expression caused by CNIs and UVA and was found to significantly inhibit GRO-α expression in keratinocytes and tumor cell lines (Schaper-Gerhardt et al., 2021, 2018). In addition, Yu et al. (2018) revealed that *CCND1* gene overexpression was most closely related to mTOR inhibitor resistance (Table 7) (Yu et al., 2018).

Epigenetic alterations (DNA methylation)

There were five articles (6% of all articles) that discussed epigenetics in cSCC among OTRs (Table 8). A comparison of RTRs who developed cSCC and those who did not identified 16 differentially methylated regions. Notable genes included *ZNF577* and *FLOT1* (Peters et al., 2018). In addition, a study by Peters et al. (2019) revealed higher DNA methylation of *SERPINB9* in RTRs who developed cSCC than those that did not. Median DNA methylation of *SERPINB9* was 58.7% (range 32.5-81.3%) for region 1 and 54.4% (30.0-78.5%) for region 2 in patients with cSCC, and 50.2% (21.8—77.5%) for region 1 and 46.4% (22.1—74.0%) for region 2 in the non-cSCC patients (region 1: $P = 0.004$; region 2: $P = 0.008$) (Table 8) (Peters et al., 2019).

DISCUSSION

This study generated a comprehensive list of 44 genes of interest and associated polymorphisms or haplotypes presently identified in the literature as having a possible association with risk of cSCC in ISPs (Table 3). Alleles identified with the greatest associated risk included genes coding for the glutathione S-transferase supergene family, which are involved in the detoxification of reactive and mutagenic compounds such as the products of UV-induced oxidative damage. We hypothesize that this increased risk is a result of decreased ability of cells to break down products of metabolic stress, in conjunction with systemic immunosuppression, providing an opportunity for neoplastic change and subsequent growth outside of immune surveillance. The nonfunctional intergenic variant polymorphism rs11820512 A allele on chromosome 11 was also highly associated with cSCC development, as well as a group of polymorphisms in the *LINC00882* gene, a nonprotein coding RNA that, when overexpressed, has been associated with a poor prognosis in hepatocellular carcinoma (Zhu et al., 2018). Haplotypes with the greatest risk for cSCC included the combination of *GSTM1 null* and the cytochrome P450 allele *CYP1A1* Val^{462}, as well as the COX-2 encoding gene *PTGS2* haplotype G_{-1195}-G_{-765}-T_{-8473}, or rs689466, rs20417, rs5275, respectively. A protective role was found for several alleles located within genes influencing pigmentation, including the brown eye allele at *OCA1/HERC2* and the C allele for *SLC45A2*, a transporter protein that mediates melanin synthesis and plays a role in skin pigmentation. OTRs homozygous for the brown eye alleles rs916977 (GG) and rs12913832 (AA) had significant delays of time to first cSCC post-transplant compared with individuals homozygous for the blue eye alleles, whereas the *SLC45A2* rs16891982 C allele was associated with a decreased HR for cSCC in univariate analysis. In contrast to the increased risk shown by *GSTM1 null*, a protective effect was found for the *GSTM1 AB* allele. Greatly reduced risk of developing cSCC was also found in those ISPs who possessed the A_{-1082}-T_{-819}-A_{-592} (IL-10 low) haplotype.

A 2016 study by Williams and Arron (2016) investigated an association between *CYP2C19* genotypes and cSCC risk in lung transplant recipients taking voriconazole, an agent commonly used to treat invasive aspergillosis in OTRs (Table 4). Although the exact mechanism of voriconazole—associated cutaneous neoplasms is unknown, this study hypothesized that a greater accumulation of voriconazole-N-oxide (VNO), a chromophore for UVB and a breakdown product of voriconazole, could lead to increased

Table 4. CNI

Study Drug	Author	Year	Study Population	Findings
CsA	Abikhair Burgo et al.	2018	Five Cell lines: • A4321: nonmetastasizing SCC from ICPs • T1: nonmetastasizing SCC from OTRs • T8: metastatic SCC from OTRs • MET1: metastatic SCC from OTRs • MET4: derived from metastasized cells of MET1	CsA exposure hastened tumor growth, upregulated IL-22R, upregulated oncogenes such as *AP-1*, and caused increased Jak1, STAT1, and STAT3 expression ($P < 0.05$).
CsA	Abikhair et al.	2016	114 OTRs with SCC, 7 SCC from OTRs and 9 SCC from ICPs, Human epidermoid carcinoma cell line A431	CsA was associated with significant risk for catastrophic SCC. SCC in OTRs treated with CsA showed increased expression of IL-22R than in ICPs. Cell lines treated with CsA showed a similar increase in IL-22R. SCC cells treated with IL-22 and CsA showed increased migratory and invasive capacity.
CsA	Arumugam et al.	2012	Human epidermoid carcinoma cell line A431	Blockade of Akt and p38 kinase-dependent signaling pathways in CsA-treated tumors abrogated growth by >90% with a decrease in proliferation and increase in apoptosis.
CsA	Dziunycz et al.	2014	SCC from 12 OTRs (treated for at least 5 y with CsA) and 19 ICPs	SCC from OTRs treated with CsA showed upregulation of ATF3 expression. CsA-treated cells showed increased ATF3 mRNA and protein expression. Skin pretreated with CsA and then exposed to UVA irradiation strongly induced ATF3 expression at both the mRNA and protein level.
CsA	Goldstein et al.	2015	Mice	CsA resulted in increased signaling through *Nfatc1*. *Nfatc1* expression was associated with increased spontaneous SCC formation.
CsA	Iotzova-Weiss et al.	2015	13 OTRs and 19 ICPs with invasive SCC, 1 OTR, and 5 ICPs with in situ SCC	CsA and prednisolone can induce S100A8/A9 expression in keratinocytes and activate NF-κB downstream.
CsA	Kuivanen et al.	2009	SCC from 20 ISPs and 20 ICPs	MMP-26 expression in cancer cells ($P = 0.04$) and that of MMP-9 in neutrophils ($P = 0.005$) were more abundant in SCCs of patients using CsA.
CsA	Sommerer et al.	2008	55 RTRs treated with CsA	Of the 55 RTRs treated with CsA, 14 developed NMSCs. NFAT-regulated gene expression (IL-2, GM-CSF, and IFN-γ) was significantly lower in patients with NMSCs.
CsA	Walsh et al.	2011	Human epidermoid carcinoma cell line A431	CsA treatment increases tumor size of xenograft human SCC. CsA treatment increased expression of the cell cycle regulatory proteins cyclin D1/3, CDK4/6, as well as VEGF. Pro-apoptotic protein Bax was decreased in CsA-treated mice, whereas Bcl-2 was increased.
CsA	Wu et al.	2010	Mice, xenografts, and cell lines	Genetic and pharmacologic suppression of calcineurin/nuclear factor of activated T cells (NFAT) promotes tumor formation. Calcineurin/NFAT inhibition counteracts p53-dependent cancer cell senescence, thereby increasing tumorigenic potential. ATF3, a member of the AP-1 family, is selectively induced by calcineurin/NFAT inhibition. Increased ATF3 expression accounts for suppression of p53-dependent senescence and enhances tumorigenic potential. Intact calcineurin/NFAT signaling is critical for p53-associated mechanisms that protect against cSCC development.
CsA	Wu et al.	2018	Mouse keratinocytes, human keratinocytes, SCC lines SCC12, 13, 15, 25, and SCC and normal skin from ISPs and ICPs	Treatment with CNI resulted in enhanced production of pro-inflammatory cytokines such as TNF-α, IL-8, and CXCL1. Treatment with CNI resulted in decreased expression of TTP, a zinc-finger protein which mediates decay of cytokine mRNA and has a tumor suppressing role ($P < 0.05$).
CsA	Xu et al.	2011	Human epidermoid carcinoma cell line A431	CsA was shown to augment tumor growth by activating TAK1, which ultimately activates NF-κB and p38 MAP kinase.
CsA and tacrolimus	Dworkin et al.	2009	Mouse models to mimic OTRs	Tacrolimus-treated mice showed a higher number of chromosomal aberrations than CsA, sirolimus, and mycophenolate. Tacrolimus and CsA showed a variation in alterations in the genome suggesting a potential for different methods of inducing SCC.

(*continued*)

Table 4. Continued

Study Drug	Author	Year	Study Population	Findings
Tacrolimus	Burke et al.	2015	10 RTRs treated with sirolimus, 11 RTRs treated with tacrolimus, and 10 ICPs	Mcl-1 expression intensity was reduced in tacrolimus-treated patients (1+ [0%], 2+ [36%], and 3+ [64%]) than in sirolimus-treated (1+ [0%], 2+ [0%], and 3+ [100%]) and ICPs (1+ [0%], 2+ [0%], and 3+ [100%]) ($P = 0.024$). No difference between groups in percentage of cells with Mcl-1 expression ($P = 1.00$), Bcl-xL expression intensity ($P = 0.134$), or the percentage of cells with Bcl-xL expression ($P = 1.00$).
Tacrolimus	Zeng et al.	2021	Mice treated with tacrolimus	IFN-γ—neutralization abrogated SCC regression, significantly reduced CD8+ T-cell infiltration into SCC, and significantly impaired the secretion of CXCL9, CXCL10, and CCL5 within the tumor microenvironment.

Abbreviations: Akt, protein kinase B; CNI, calcineurin inhibitor; CsA, cyclosporin A; ICP, immunocompetent patient; ISP, immunosuppressed patient; MAP, mitogen-activated protein; MMP, matrix metalloproteinase; NMSC, nonmelanoma skin cancer; OTR, organ-transplant recipient; RTR, renal transplant recipient; SCC, squamous cell carcinoma; STAT, signal transducer and activator of transcription; TAK1, TGFβ-activated kinase 1

A summary of studies examining mechanisms underlying the protumorigenic effects of systemic CNIs.

voriconazole—associated cSCC. Allelic variant *17 (rs12248560) found in rapid metabolizers of voriconazole showed a 74% increased hazard for cSCC in these patients, presumed to be related to the increased accumulation of VNO (Williams and Arron, 2016). Considering this fact, clinicians typically suggest that alternative antifungal agents be used where possible among this population. Overall, the identified polymorphisms and haplotypes confirm a genetic component and predisposition in the development of cSCC among OTRs. Understanding these risks and identifying relevant polymorphisms in OTRs could allow for identification and closer surveillance of high-risk patients and ultimately improve morbidity and mortality among this population.

Thirty-eight percent of articles focused on somatic gene mutations or mRNA/protein expression within cSCC tumors in ISPs (Tables 1 and 2). The *CDKN2A* gene encodes both $p14^{ARF}$ and $p16^{INK4A}$ and is commonly mutated in cSCC (Lobl et al., 2021). These proteins act as tumor suppressor genes and regulators of the p53 and Rb pathways, respectively. This review included two studies that evaluated chromosomal inactivation at 9p21-22, an area that includes the tumor suppressors $p16^{INK4A}$ and $p14^{ARF}$ (Tables 3 and 8). DNA methylation at this location was shown to be the most common mechanism of inactivation in cSCC, irrespective of immune status. Interestingly, OTRs showed significantly lesser genetic and epigenetic inactivating events at 9p21-22 than ICPs, and according to the authors, the explanation for these findings remains elusive (Table 8) (Brown et al., 2004). A separate study found broadly reduced allelic balance at 9p21-22 in both OTRs and cSCC; however, a unique microsatellite location, D9S162, was identified in OTRs that showed reduced allelic balance when compared with ICPs (Table 3) (Mühleisen et al., 2012). Taken together, considering that OTRs have a greater risk of developing cSCC despite showing fewer genetic and epigenetic inactivating events at 9p21-22, reduced allelic balance at D9S162 could contribute to the increased incidence and more aggressive carcinogenesis of cSCC in OTRs. Although the underlying cause of this reduction remains unknown, it is pertinent to also consider the effect of reduced immune surveillance in ISPs that may allow for tumors with fewer mutations to proliferate.

Although the literature suggests p14 and p16 may contribute to the development of cSCC in OTRs, the role of p53 expression, a commonly upregulated and sometimes mutated tumor suppressor, is more controversial. Although some studies found increased staining intensity of p53 within OTRs, others found that expression of both mRNA and protein for p14, p16, and p53 is independent of immune status (Table 1) (de Graaf et al., 2008; Gutiérrez-Dalmau et al., 2010; Küsters-Vandevelde et al., 2009; Seçkin et al., 2002). Blokx et al. (2003) showed higher prevalence of p53-negative tumors in cSCC from RTRs than in ICP tumors (30% vs. 0%) (Table 1). One possible explanation was discovered by Maurer et al. (1997), who examined both UV-exposed and UV-protected skin in patients with HIV and found that a significant positive correlation existed between the amount of sun exposure and the amount of p53 staining seen in adjacent epidermal tissue (Table 2) (Maurer et al., 1997). This lack of congruity involving p53 expression is likely multifactorial and could be a result of many factors such as differences in immunosuppressive regimen, smoking status, and UV exposure, among others. Further clarity might be provided by studies with larger sample sizes, assessing both p53 expression and mutational status.

There is a well-known role of altered miRNA expression causing gene dysfunction within tumors. Of the examined miRNAs in OTRs, miR-135b was the most upregulated, by 21.5-fold in OTRs and 13.3-fold in ICPs ($P = 0.0001$) (Table 1) (Olasz et al., 2015). Upregulation of miR-135b ultimately resulted in increased tumor growth, motility, and invasiveness in several types of tumors, including cSCC (Olasz et al., 2015). Identification of miR-135b in OTRs could potentially be used by clinicians during routine surveillance of OTRs. These miRNAs can suppress protein translation and regulate gene expression post-transcriptionally. In addition, miRNA plays a role in modulating the immune response, particularly among OTRs (Sarma et al., 2012).

Table 5. AZA

Author	Year	Study Population	Findings
Brem et al.	2010	HaCaT keratinocyte cell line	Interaction between low doses of UVA and AZA causes DNA single-and double-strand breaks. AZA/UVA-induced DNA lesions provoke canonical DNA damage and activate the ATM/Chk2 and ATR/Chk1 pathways. Higher levels of photochemical DNA damage induce a proteasome-mediated degradation of Chk1 and checkpoint abrogation.
Inman et al.	2018	30 SCCs from ISPs and 7 SCCs from ICPs	Signature 32 found to be a mutational signature unique to ISPs receiving AZA. Signature 32 was responsible for 65% of the significantly mutated genes in the SCC samples, including NOTCH1/2, TP53, and CDKN2A.
Kalra et al.	2012	AZA-treated mice	A robust systemic induction of the Keap1/Nrf2/ARE pathway protects cells with 6-thioguanine incorporations against oxidative stress caused by UVA radiation.
Kalra et al.	2011	AZA-treated mice and non-treated mice	Genetic upregulation of Keap1/Nrf2/ARE reduces incorporation of 6-thioguanine in DNA after treatment with AZA.
O'Donovan et al.	2005	Normal skin from three AZA-treated patients and three without treatment, normal skin in five patients newly started on AZA than to skin from the same patients before treatment	Skin from AZA-treated patients showed 6-thioguanine representing around 0.02% substitution of DNA guanine, whereas those without treatment had no 6-thioguanine incorporation. AZA treatment caused a significant reduction in the minimal erythema dose for UVA ($P = 0.025$ by paired *t*-test compared with the pretreatment value).
Perrett et al.	2010	52 SCCs from OTRs treated with AZA and 34 SCCs from ICPs	MSH2 and MLH1 protein expression was not altered in SCCs from OTRs on AZA and there was no difference in expression levels between SCCs from OTRs and ICPs.

Abbreviations: AZA, azathioprine; ICP, immunocompetent patient; ISP, immunosuppressed patient; OTR, organ-transplant recipient; SCC, squamous cell carcinoma.

A summary of the genetic mutations, polymorphisms, and expression alterations that increase risk of cSCC in patients taking AZA.

Table 6. Voriconazole

Author	Year	Study Population	Findings
Williams and Arron	2016	A total of 177 lung transplant recipients who developed SCC after taking voriconazole	Voriconazole was associated with a 73% increased risk for SCC development in lung transplant recipients. Allelic variant *17 of *CYP2C19* had a 74% increased hazard for SCC (95% CI = 1.06–2.84; $P = 0.03$).

Abbreviations: CI, confidence interval; cSCC, cutaneous squamous cell carcinoma; SCC, squamous cell carcinoma.

A summary of the genetic mutations, polymorphisms, and expression alterations that increase risk of cSCC in patients taking voriconazole.

Table 7. mTOR Inhibitors

Author	Year	Study Population	Findings
Koletsa et al.	2018	23 SCCs from OTRs before or after switch to mTOR inhibitor	mTOR inhibition did not significantly change the immunohistochemical expression of molecules upstream of mTOR (p-mTOR, PI3K, p-Akt).
Schaper-Gerhardt et al.	2018	Human epidermoid carcinoma cell line A431, SCC 12/13 cell lines, and organotypic skin models	Sirolimus downregulated the expression of the oncogene *ATF3* that is commonly induced by CsA and UV light.
Schaper-Gerhardt et al.	2021	Neonatal normal human epidermal keratinocytes, human epidermoid carcinoma cell line A431, SCC 12/13 cell lines, and organotypic skin models	Sirolimus significantly inhibited GRO-α expression in keratinocytes and tumor cell lines and decreased the expression of the corresponding receptor CXCR2.
Yu et al.	2018	Everolimus-sensitive (HSC-1) and everolimus-resistant (A431) SCC cell lines	*CCND1* gene overexpression was most closely related to mTOR inhibitor resistance. *MYC/CCND1/TP73/NUPR1/SBD/ERBB2/CDKN2B* genes were all related to mTOR inhibitor resistance.

Abbreviations: CsA, cyclosporin A; OTR, organ-transplant recipient; p-Akt, phosphorylated protein kinase B; p-mTOR, phosphorylated mTOR; PI3K, phosphatidylinositol 3-kinase; SCC, squamous cell carcinoma.

A summary of the genetic mutations, polymorphisms, and expression alterations that increase risk of SCC in patients taking mTOR inhibitors.

This review identified many reports of drug-induced genetic alterations associated with immunosuppressive medications in OTRs (Tables 4–7). AZA has been linked to the development of cSCC in ISPs (Table 5). AZA use has been shown to lead to the accumulation of 6-TG in cellular DNA, which has a greater absorption potential for UVA than normal DNA, and thus increased development of cutaneous malignancies, while also reducing DNA repair activity (de Graaf et al., 2008; O'Donovan et al., 2005). Recently, a previously unknown mutational signature in cSCC of OTRs, termed signature 32, was discovered during whole exome sequencing and mutational signature analysis of OTRs. Analysis of treatment times revealed a strong positive correlation with the estimated time of AZA exposure and the prevalence of signature 32 (Table 5) (Inman et al., 2018). These findings indicate that although AZA may prevent

Table 8. Epigenetic Alterations

Author	Year	Study Population	Findings
Brown et al.	2004	30 SCCs from ISPs and 10 SCCs from ICPs	SCCs in ISPs showed fewer epigenetic inactivating events at p14 and p16 when compared with ICPs.
Laing et al.	2010	47 SCCs and 40 normal skin samples from RTRs	SCC was hypomethylated compared with adjacent non-neoplastic skin.
Peters et al.	2018	27 RTRs with SCC and 27 RTRs without SCC	Identified 16 differentially methylated regions in RTRs including ZNF577, a zing-finger protein, and FLOT1, a protein involved in T-cell migration.
Peters et al.	2019	Cohort 1: 19 RTRs with cSCC and 19 RTRs without cSCC Cohort 2: 45 RTRs with cSCC and 37 RTRs without cSCC	Higher DNA methylation of *SERPINB9* in RTRs who developed cSCC than those that did not. Median DNA methylation of *SERPINB9* was 58.7% (range: 32.5−81.3%) for region 1 and 54.4% (30.0−78.5%) for region 2 in patients with cSCC and 50.2% (21.8−77.5%) for region 1 and 46.4% (22.1−74.0%) for region 2 in the non-cSCC patients (region 1: $P = 0{\cdot}004$ and region 2: $P = 0.008$).
Sherston et al.	2014	57 RTRs with SCC and 49 RTRs without SCC	*TSDR* methylation analysis also showed an association between high Treg levels in RTRs with SCC compared with those without.

Abbreviations: cSCC, cutaneous squamous cell carcinoma; ICP, immunocompetent patient; ISP, immunosuppressed patient; RTR, renal transplant recipient; SCC, squamous cell carcinoma; Treg, regulatory T cell.

A summary of epigenetic alterations discovered in ISPs with an associated risk of SCC development.

organ-transplant rejection, it may actively lead to the development of cutaneous malignancy.

Commonly used transplant medications also lead to changes in the transcriptional profile that can influence tumor phenotype. CNIs, in particular, have been linked to multiple potential methods of inducing and promoting cSCC. Several studies examined the relationship between CNIs and proinflammatory cytokines and their downstream pathways in the pathogenesis of cSCC (Table 4). Treatment with CsA, a frequently used CNI, was shown to hasten tumor growth, upregulate IL-22R expression, and cause increased Jak1, STAT1, and STAT3 expression (Abikhair Burgo et al., 2018). Upregulation of IL-22R has a strong association with the development of catastrophic cSCC, which is defined as multiple primary tumors manifesting at the same time in the same patient (Abikhair et al., 2016). CsA was also shown to enhance production of proinflammatory cytokines by decreasing expression of tristetraprolin, a tumor suppressor that negatively controls production of proinflammatory cytokines (Wu et al., 2018). Another pathway by which CsA induces a proinflammatory response is through phosphorylation of TAK1, which ultimately activates NF-κB, resulting in enhanced proliferation and reduced apoptosis (Xu et al., 2011). The last group of studies involved *ATF3*, an oncogene and member of the AP-1 family that has been linked with cSCC development (Wang et al., 2007). ATF3 was shown to be selectively induced by CNIs (Wu et al., 2010). In addition, an additive effect was found between CsA and UVA, which together induce ATF3 expression through a direct drug effect and UVA-induced ROS formation (Table 4) (Dziunycz et al., 2014).

This review also identified a role for epigenetic alterations, such as DNA methylation alterations, in the development of cSCC among OTRs (Table 8). Both hypomethylation and hypermethylation have previously been associated with the development of skin cancer in ICPs (Sigalotti et al., 2002; Tyler et al., 2003). A comparison of RTRs who developed cSCC and those who did not identified 16 differentially methylated regions. Notable genes included *ZNF577*, coding for a zinc-finger protein, and *FLOT1*, coding for a protein involved in T-cell migration (Peters et al., 2018). Because of the important adaptive immune response to neoplastic growth, it follows that alterations in *ZNF577* and T-cell migration could predispose patients to cSCC development. Clinicians should be aware of the potential for testing methylation patterns because this could lead to an effective method of stratifying pretransplant patients according to future risk of cSCC development. Overall, very little data exists regarding epigenetic alterations among OTRs with cSCC. Overall, epigenetic changes such as altered DNA methylation patterns indicate additional potential factors contributing to the high incidence and morbidity of cSCC in OTRs while also providing possible avenues of pretransplant risk stratification, post-transplant surveillance, and treatment selection to improve outcomes.

Furthermore, one study focused on a unique area that will require further research. Speeckaert et al. (2012) reported a significant association of the haptoglobin phenotype Hp 1-1 with the risk of cSCC (Table 3) (Speeckaert et al., 2012). Considering this increased long-term risk of cSCC, patients with this phenotype have an increased need of preventive measures including self-skin exams, regular visits to a dermatologist, and thorough UV-protection habits.

A limitation to this review was the exclusion of studies focusing solely on the ICP population. The goal of this review was to gather all studies to date that have focused on tumor profiling in the ISP population. Although it is necessary to understand these same tumor characteristics in the ICP population, the studies included in this review highlighted the direct comparisons between the ICPs and ISPs as well as studies comparing ISPs with and without cSCC. Significant work has been done in ICP populations with notable findings including the significant roles of TP53, NOTCH, TGFβ, and CDKN2A in the development of cSCCs in this population (Al-Rohil et al., 2016; Chitsazzadeh et al., 2016; Ji et al., 2020; Lazo de la Vega et al., 2020; Sarin et al., 2020; Thomson et al., 2021; Zheng et al., 2021). Review of these important studies did not alter the conclusions in this study. However, future studies may more closely examine any differences in these key pathways between the ISP and ICP populations, with a focus on using advanced approaches such as single cell transcriptomics.

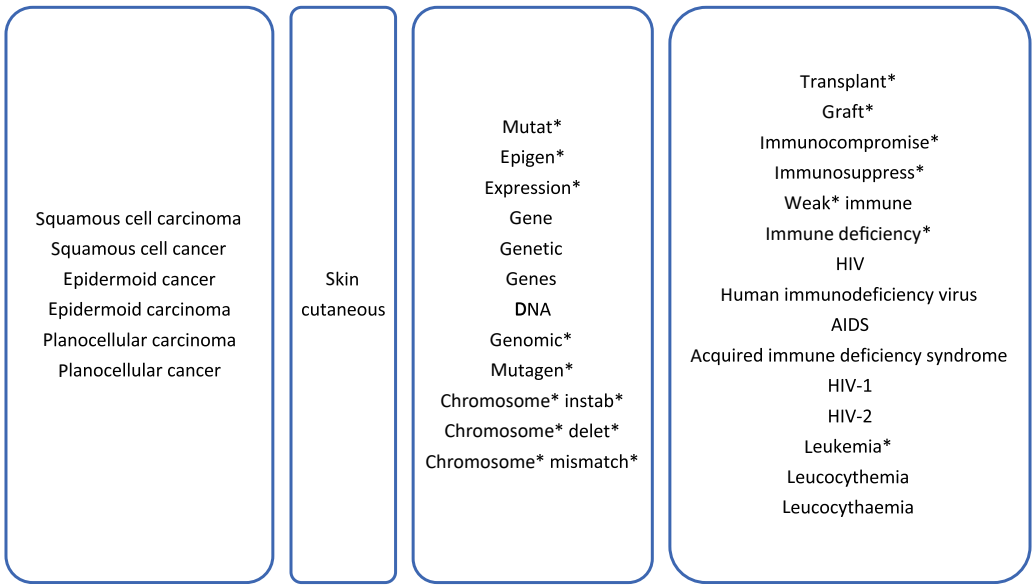

Figure 2. Search terms for database query. Representation of search terms used in the database search. Search terms within each box were connected by the Boolean operator OR, whereas each box represents a main search domain connected by the Boolean operator AND. Asterisk (*) utilized as wildcard symbol to broaden search by finding words that start with the same letters.

CONCLUSION AND FUTURE DIRECTIONS

To our knowledge, this is the first systematic review of the genetic, epigenetic, transcriptional, and translational alterations associated with cSCC arising in ISPs compared with ICPs. ISPs are a growing group with known increased risk of cSCC and limited treatment options, specifically for late-stage disease. Understanding the drivers of cSCC in this population is an important step to reducing morbidity and mortality in this high-risk population.

Dermatologists are faced with the challenge of identifying which patients are at increased risk for SCC to determine appropriate screening intervals and preventive strategies. Understanding a patient's risk may also inform the approach to pharmacologic immunosuppression for solid OTRs and other patients on chronic immunosuppressive medications. Mutations in the genes coding for the glutathione S-transferase family of proteins (*GSTM1*, *GSTM3*, and *GSTP1*), and the gene coding for IRF4 were shown in this review to modulate the risk for NMSC in OTRs. This may serve as an additional tool for patient risk stratification for dermatologists and physicians prescribing immunosuppression.

Treatment is another important challenge, specifically in patients with advanced disease of the head and neck. Currently available systemic treatments have poor response rates and there are few options, especially for OTRs that are not eligible for checkpoint inhibitor therapy. This review highlights potential therapeutic targets that warrant further exploration such as Bcl-xL.

Recently, techniques such as single cell RNA sequencing and spatial transcriptomics have emerged that allow high-definition profiling of tumors. Proteomic and metabolomic approaches also provide valuable phenotypic information, specifically regarding pathways that may be pharmacologically targeted. Studies using these technologies to compare tumors from ISPs to ICPs should be performed to improve the understanding of cSCC carcinogenesis in these patients, leading to improved targeted therapies.

MATERIALS AND METHODS

This review was conducted according to the 2009 preferred reporting items for systematic reviews and meta-analyses guidelines (Moher et al., 2009). A literature search was conducted on 1 November 2021 in MEDLINE, Cinahl, and Scopus using key phrases. Four main search domains were used, which were combined with the Boolean operator AND, whereas search terms contained within each domain were combined with the Boolean operator OR (Figure 2).

The retrieved literature was screened by title and abstract for inclusion. If the suitability of an article was unclear, the full text was assessed. Studies were selected if they met the following inclusion criteria: i) English language, ii) focus on cSCC, iii) study sample of ISPs, OTRs, or representative model, iv) data focusing on epigenetic or genetic alterations or gene expression, and v) full text available. Articles were excluded if: i) original data were not reported, ii) no data was reported regarding ISPs or OTRs, iii) articles solely reported isolated cases, iv) articles did not discuss genetic or epigenetic alterations or gene expression, iv) data were not reported for cSCC, or vi) the article was not written in the English language. Human cell lines and animal models representative of human ISPs were not excluded. Additional articles discovered during the completion of full-text review of the selected articles were added to the review if they met the inclusion criteria, did not meet exclusion criteria, and were not duplicates.

Data availability statement

We do not have any additional data or materials to share.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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