Published in final edited form as:

Clin Lab Med. 2019 March ; 39(1): 171–183. doi:10.1016/j.cl.2018.10.006.

Signaling Molecules in Post-Transplantation Cancer

Murugabaskar Balan, PhD,

Instructor, Division of Nephrology, Boston Children's Hospital, Boston, MA, USA

Samik Chakraborty, PhD, and

Research Fellow, Division of Nephrology, Boston Children's Hospital, Boston, MA, USA

Soumitro Pal, PhD

Associate Professor, Division of Nephrology, Boston Children's Hospital, Boston, MA, USA

Summary:

Incidence and mortality of cancers among transplant recipients are at least two-fold higher when compared with the general population. This increased risk for cancer can be attributed to the burden of immunosuppressive regimens, infection, and altered immune phenotype. We have summarized the molecular mechanisms associated with the direct influence of immunosuppressive agents on cell-intrinsic tumorigenic pathways. Future research and clinical studies will reveal the efficacy of novel immunosuppressive agents (like JAK inhibitor) and the success of development of new concepts of immunomodulatory strategies, such as targeting T cell glycolysis, T cell co-signaling molecules and purinergic signaling axis. The goal is to achieve long-term graft survival while mitigating the risk of post-transplantation cancer.

Keywords

Post-transplantation cancer; CNI; immunosuppression; risk factor; signaling mechanisms

Introduction

In solid organ transplantation (SOT), the short-term graft survival rate has significantly increased with the use of novel and better immunosuppressive agents. However, advent of

Address for Correspondence: Soumitro Pal, Division of Nephrology, Children's Hospital, 300 Longwood Ave, Boston, MA 02115; soumitro.pal@childrens.harvard.edu, Phone: 617-919-2989, Fax: (617) 730-0365.

Author Contact Information

Murugabaskar Balan, Division of Nephrology, Children's Hospital, 300 Longwood Ave, Boston, MA 02115;

murugabaskar.balan@childrens.harvard.edu

Samik Chakraborty, Division of Nephrology, Children's Hospital, 300 Longwood Ave, Boston, MA 02115;

samik.chakraborty@childrens.harvard.edu

Soumitro Pal, Division of Nephrology, Children's Hospital, 300 Longwood Ave, Boston, MA 02115;

soumitro.pal@childrens.harvard.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

MB and SP are co-senior authors.

Disclosure Statement

The authors have nothing to disclose.

malignancies after SOT drastically affects the long-term survival of the graft and the life expectancy of the recipients¹. Epidemiological studies from different regions of the world reveal that cancer development among transplant recipients is one of the major adverse outcomes after SOT and cancer-related mortality rates are much higher among organ transplant recipients². For instance, a nested case-control study of kidney transplant recipients indicated that the major reason for deaths in patients with a functional renal graft was cancer³. Unfortunately, the second and fourth most common cause of death among liver and lung transplant recipients, respectively, is cancer⁴.

In the US, a large-scale registry linkage study, comparing data from the Scientific Registry of Transplant Recipients (SRTR) (a federal registry) with several state and regional cancer registries, indicated a wide spectrum of cancer risk for 32 different malignancies among organ transplant recipients⁵. The study measured the relative risk of cancer in transplant recipients as Standardized Incidence Ratio (SIR) (i.e., observed/expected cases), a statistical measure to compare the relative risk of cancer development among transplant recipients compared with the general population. The SIR risk measurements among kidney, liver, heart, and lung transplant recipients (among 175732 transplants comprising 39.7% of US total during 1987-2008) revealed that cancer etiology and the type of cancer developed varies according to the organ transplanted. For example, liver cancer risk increased only among liver transplant recipients, whereas increased risk of kidney cancer was observed even among liver and heart transplant recipients⁵. Excluding squamous cell and basal cell skin cancer types, for which data are not collected by the cancer registries, the study observed a 2- fold overall increased risk of cancer among transplant recipients⁵.

The most common post-transplantation malignancy with increased risk was Non-Hodgkin's Lymphoma (NHL) with SIR of 7.54. The SIRs of the cancers of the lung and kidney were 1.97 and 4.65, respectively. Notably, renal cell carcinoma (RCC) or kidney cancer risk during the first year after kidney transplant was much higher with an SIR in the range of 7.28-10.28, indicating an accelerated risk of malignant transformation of the transplanted organ⁵. The SIRs for other cancers including liver, colorectal cancer, thyroid cancer, and myeloid neoplasms were also higher⁵.

Apart from known risk factors, such as recipient age, previous cancer, sun exposure (UV light) and donor-transmitted cancer, viral infections-related malignancies, such as NHL (Epstein-Barr virus [EBV]), Kaposi sarcoma (human herpesvirus 8 [HHV 8]) and liver cancer (Hepatitis B and C viruses [HBV/HCV]) have a higher rate of occurrence in organ transplant recipients². Immunosuppression impairs the ability of the host immune system to control viral infection and reduces tumor immunosurveillance. Conceivably, the major risk factor for post-transplantation cancer arises from the indispensable need for long-term immunosuppression after SOT.

Interestingly, recent reports suggest that immunosuppressive agents can activate tumorigenic pathways independent of the involvement of the host immune system. It has also been reported that with the use of immunosuppressive agents, preexisting cancer progress at much higher rate in transplant recipients². Further, the life-long need for immunosuppressive agents to sustain the transplant limits the treatment options available for post-transplant

cancer. This is because the tumorigenic pathways directly activated by immunosuppressive agents can potentially interfere with the efficacy of anti-cancer agents used to treat transplant recipients with cancer. The molecular mechanisms associated with the “cancerous” side effect of immunosuppressive agents are not completely understood. Therefore, a better understanding of the tumorigenic mechanisms that can solely be attributed to immunosuppressive agents will help us define more effective cancer-treatment strategies for organ transplant recipients.

In this review, we shed light on the cell-intrinsic tumorigenic pathways directly activated by immunosuppressive agents and the infection- and immune-mediated mechanisms of cancer development in organ transplant recipients.

Hypoxia-related signaling molecules in post-transplantation cancer:

In the process of procurement, preservation, and reperfusion, the donor organs are subjected to hypoxia (lack of oxygen supply) and ischemic injury (lack of blood supply), commonly known as Ischemia-reperfusion injury (IRI). Hypoxia and IRI are detrimental for preserving the organ function and long-term graft survival⁶. The hypoxia-related signaling molecules involved in tumorigenic pathways are shown in Figure 1.

In response to hypoxia, oxygen-sensitive transcription factors, termed as hypoxia-inducible factors (HIFs), regulate the transcription of hypoxia-responsive genes as a protective mechanism. A hypoxia regulated α chain (1 α , 2 α , and 3 α), bound to an oxygen-independent β chain makes up the heterodimer complex of HIF. The cytoprotective function of HIF-1 α and HIF-2 α are well studied. Activity of HIFs increases the expression of cytoprotective molecules including heat shock proteins, erythropoietin, and heme oxygenase-1 (HO-1). HO-1 catabolizes heme and the byproducts of this enzymatic reaction mediate cytoprotection⁷. Reports suggest that HO-1 promotes anti-apoptotic pathways in response to IRI⁸ and may play a significant role in the induction of tumorigenic pathways. Another molecule, vascular endothelial growth factor (VEGF), a downstream target of HIFs, also can contribute to tumor development when its level rises, given that it plays a critical role in the formation of new blood vessels in tumor (tumor angiogenesis)⁹.

Under normoxic conditions, the subunits of HIFs are targeted for polyubiquitination- and proteasome-mediated degradation by the tumor suppressor protein Von Hippel-Lindau (VHL)⁶. VHL inactivation represents a pseudo-hypoxic condition and causes constitutive activation of HIFs, leading to increased expression of cytoprotective molecules. Interestingly, germline mutations in VHL gene (known as VHL disease) are associated with abnormal regulation of hypoxic response genes and predisposition to many malignancies including renal tumors, implying the role of hypoxia-related signaling pathways in post-transplantation cancer.

Reactive oxygen species (ROS) (such as superoxide, peroxide and hydrogen peroxide) are reactive molecules derived from molecular oxygen and hydroxyl-radical as a result of mitochondrial electron transport chain and the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. Increase in ROS during IRI or pharmacological agent-induced generation of ROS leads to oxidative stress and cell death⁸. However, cellular anti-

oxidant enzymes, such as HO-1, manganese superoxide dismutase (MnSOD) and NAD(P)H dehydrogenase [quinone] 1 (NQO1), counteract ROS and mediate cytoprotection. Thus, a critical balance exists between the levels of ROS and the anti-oxidant system; and a shift towards increased expression of cellular anti-oxidants can lead to oncogenic transformation of the cell⁷.

Molecular targets of immunosuppressive agents:

Immunosuppressive regimens are essential in SOT to suppress alloimmunity and prevent graft rejection. Understanding the molecular targets of immunosuppressive agents can shed light on pro-tumorigenic mechanisms associated with immunosuppression. Effector T cells play a critical role in the immunological rejection of transplanted graft. Immunosuppressive agents, used at various levels and combinations in SOT, inhibit the stimulation, activation, and expansion of effector T cells through different mechanisms.

Target genes of transcription factor nuclear factor of activated T cells (NFAT) are essential and contribute to the effector functions of T cell. T cell receptor (TCR) signaling increases intracellular calcium levels and activates calmodulin, which in turn binds and activates calcineurin. Phosphorylated NFAT is inactive and resides in the cytoplasm. Activated calcineurin, with its protein phosphatase activity, dephosphorylates NFAT and promotes the nuclear translocation of NFAT to activate transcription of NFAT target genes, including IL-2, IL-4 and Interferon (IFN)- γ , leading to T cell activation¹⁰.

Calcineurin Inhibitors (CNIs): CNIs such as Tacrolimus (TAC, FK506) and Cyclosporin A (CsA), the most widely used immunosuppressive agent in transplant medicine, inhibit the activity of calcineurin, thereby suppressing NFAT-mediated transcription of cytokines necessary for the activation of T cells. Immunophilins such as cyclophilins and FK506-binding proteins (FKBPs) are cytosolic peptidyl-prolyl isomerases (PPIs) that catalyze *cis-trans* isomerization of proline residues and act as chaperones (proteins that regulate inter- and intra-molecular interactions and govern proper protein folding and complex formation). CsA and TAC form drug/immunophilin complexes by binding to cyclophilin and FKBP, respectively, and inactivate calcineurin¹⁰.

mTOR inhibitors: The mechanistic target of rapamycin (mTOR) signaling pathway regulates cell cycle progression, metabolism and protein synthesis in response to a variety of stimuli including growth factors and nutrients. mTOR is a serine/threonine protein kinase and downstream effector molecule for growth factors-induced Phosphoinositide 3-kinase (PI3K)-Akt signaling pathways. The tumor suppressor gene phosphatase and tensin homolog (PTEN) inhibits Akt activation through PI3K suppression and PTEN is lost or mutated in many cancer types, leading to hyperactivation of mTOR pathway in cancer cells.

mTOR forms two distinct signaling complexes namely complex 1 (mTORC1), containing RAPTOR, and complex 2 (mTORC2), containing RICTOR. mTOR complexes regulate protein translation by modulating the activity of S6 kinase (known as p70S6K) and eukaryotic translation initiation factor 4E (eIF4E) (through the regulation of eIF4E-binding protein (4E-BP). mTOR inhibitors, such as Rapamycin (Sirolimus) and its derivatives (Temsirolimus and Everolimus), form drug/immunophilin complex by binding to FKBP12

and inhibit mTOR. IL-2/IL-2 receptor (IL-2R)- and other growth factors-mediated signaling trigger mTOR pathway in T cells. mTOR inhibitors affect T cell protein synthesis, leading to T cell inactivation¹⁰.

Purine analogues: Impeding DNA synthesis inhibits cell proliferation. Guanosine, a purine nucleoside, is an essential building block of DNA and RNA. Inosine-5'-monophosphate dehydrogenase (IMPDH) is the enzyme involved in the *de novo* synthesis of guanosine. In contrast to many other cell types, which can also synthesize guanosine by recycled salvage pathways, T-lymphocytes are dependent on IMPDH for *de novo* synthesis of guanosine. Therefore, molecules (Micophenolate mofetil [MMF] and Micophenolic acid [MPA]) that inhibit IMPDH activity and disrupt guanosine synthesis or purine analogues that interfere with the incorporation of endogenous purines e.g. (Azathioprine [AZA], 6-mercaptopurine [6-MP]) inhibit T cell proliferation¹⁰.

Biologics: Biologic medicines, such as monoclonal antibodies, polyclonal antibodies and recombinant fusion proteins, targeting key pathways involved in T cell activation, are predominantly used either for induction therapy (first phase of treatment) or in the treatment of acute rejection. Antibodies targeting IL2-R (Anti-IL-2R antibody, Basiliximab) have remarkable success in preventing graft rejection. Biologics targeting co-stimulatory pathways of T cell activation are promising medicines in SOT. B7 family of ligands can bind to both CD28 and cytotoxic T-lymphocyte associated protein 4 (CTLA)-4. In contrast to B7-CD28 interactions, which promote co-stimulatory signal, B7-CTLA-4 interactions inhibit T cell activation. A recombinant CTLA-4 linked to an immunoglobulin frame, comparable to the soluble form of CTLA-4, can disrupt the B7-CD28 co-stimulatory interactions and inhibit T cell activation¹¹.

The mechanistic targets of different immunosuppressive agents used in SOT are depicted in Figure 2. As discussed above, biologics are mostly used in induction therapy, and among pharmacological immunosuppressive agents, CNIs have been the standard of care in maintenance immunosuppression after SOT. However, malignancy is one of the important risks associated with the use of immunosuppressive agents including CNIs¹².

Immunosuppressive agent-induced cell-intrinsic tumorigenic pathways:

Recent reports suggest that immunosuppressive agents can directly promote tumorigenic pathways. The molecular events related to the immunosuppressive agent-induced growth and proliferation of cancer cells are represented in Figure 3.

Role of oncogene Ras: Activated Ras proteins transmit signals to other kinases including Raf family of kinases, resulting in the activation of mitogen activated protein kinase (MAPK) and extracellular-signal-regulated kinase (ERK). In addition to Raf, Ras also mediates its action through the activation of Rho and PI3K pathways. Therefore, sustained activation of Ras can transduce mitogenic signals and promote the growth of cancer cells. Raf kinase inhibitory protein (RKIP), an inhibitor of Raf kinase, suppresses Ras-Raf pathway. Carabin is an endogenous inhibitor of Ras. Interestingly, CNI treatment inhibits

both RKIP and carabin in cancer cells, leading to hyper-activation of Ras-Raf pathway and promotion of tumor growth¹³.

Receptor Tyrosine Kinases (RTKs)-mediated signaling: RTKs are cell surface receptors that bind and respond to various factors, such as chemokines, cytokines and other growth factors (such as Epidermal Growth Factor [EGF], Insulin, and Hepatocyte Growth Factor [HGF]). RTKs play an important role in the regulation of cell growth, differentiation and survival. Ligand binding to the extracellular domains of the RTKs induces cross-phosphorylation of specific tyrosine residues in the intracellular domains of the RTKs. Phosphotyrosine residues thus generated serve as docking sites of several Src Homology 2 (SH2) domain containing signal transduction adapter molecules, which in turn relay the signaling to diverse downstream intracellular signaling pathways including Ras-Raf-MAPK and PI3K-Akt signaling pathways⁷.

RTK c-MET and its ligand HGF play a significant role in the growth and progression of cancer cells. Transcription factors nuclear factor erythroid 2 [NF-E2]-related factor 2 (Nrf2) and BTB and CNC homology 1 (Bach1) regulate HO-1 expression in a positive and negative manner, respectively. In renal cancer cells, CNI treatment induces nuclear localization of Nrf2, leading to overexpression of HO-1 and plays an important role in the growth and survival of cancer cells⁷.

Modulation of chemokine receptor expression: Chemokines and their receptors play an important role in chemotaxis. Recent studies suggest a role in tumor growth as well. Chemokine receptor C-X-C Motif Chemokine Receptor 3 (CXCR3), belonging to the family of G-protein-coupled-receptors (GPCRs), exists as two isoforms namely CXCR3-A and CXCR3-B¹⁴. In contrast to CXCR3-A-mediated signaling, which promotes proliferative signals, CXCR3-B-mediated signaling inhibits the proliferation of cancer cells. In cancer cells, CNI treatment downregulated the expression of CXCR3-B isoform, whereas expression of CXCR3-A was not affected¹⁴. This report suggests that CNI-mediated modulation of alternative splicing of CXCR3 gene can play a significant role in the growth of tumors¹⁴.

Overexpression of VEGF: Unphosphorylated proline-rich Akt substrate 40 (PRAS40) negatively regulates mTORC1 activity by sequestering RAPTOR from the complex. CNI-induced Ras activation promotes phosphorylation of PRAS40 through protein kinase C (PKC) and relieves the negative effect of PRAS40 on mTOR complex, leading to transcriptional activation of mTOR downstream target genes including VEGF. Thus, CNI-induced crosstalk between Ras and mTOR increases the expression of VEGF and promotes rapid progression of tumor¹⁵.

Role of cytokines: It is well established that cytokines, such as Interleukins and Interferons, modulate tumorigenic pathways. A notable cytokine involved in tumorigenesis is Interleukin 6 (IL-6). The tumorigenic role of Janus Tyrosine Kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) activation in response to IL-6 has been well described in many cancer types. CNI treatment increases IL-6 production in EBV-infected B cells and can play a potent role in cancer development¹.

Transforming growth factor beta (TGF- β), a multifunctional polypeptide belonging to the TGF- β family of cytokines, while acting as a tumor suppressor during primary tumor development, promotes cancer cell migration and stimulates epithelial-mesenchymal transition, an invasive phenotype associated with metastasis. Reports suggest that CNI-induced TGF- β secretion can impart an invasive phenotype to non-transformed cells and promote tumor growth¹⁶.

Role of p53: p53, a tumor suppressor, induces cell cycle arrest and apoptosis in response to variety of stimuli, including UV radiation (sunlight exposure), which cause DNA damage. CNIs downmodulate p53-dependent cell senescence in some cell types. Activating transcription factor 3 (ATF3) is negatively regulated by calcineurin/NFAT pathway; and ATF3 suppresses p53-dependent cell senescence. CNI-mediated inhibition of calcineurin/NFAT increases the expression of ATF3 and inhibits p53-dependent apoptotic pathways in cancer cells¹⁰.

Anti-cancer effects of mTOR inhibitors: Although relatively less potent in terms of immunosuppression, reports suggest that the use of mTOR inhibitors have potent anti-cancer effects as evident from the decreased risk of cancer in transplant recipients treated with mTOR inhibitors (like Rapamycin), either before or after CNI regimen¹². However, mTOR inhibition leads to the withdrawal of the negative regulatory loop between p70S6K and Insulin Receptor Substrate 1 (IRS-1), resulting in IRS-1-mediated Akt activation. Also, mTORC2, which is mostly insensitive to Rapamycin, can contribute to Akt activation. More research is needed to elucidate the tumor resistance mechanisms associated with mTOR inhibition¹².

Oncogenic viral infections in post-transplantation malignancies:

Opportunistic and co-tumorigenic viral infections, such as EBV, HHV8 and Human papillomavirus (HPV), can promote certain types of cancer due to immunosuppression in a transplant recipient. Cytomegalovirus (CMV) infection, the most prevalent infection following transplantation, can lead to CMV syndrome and end-organ disease. Although CMV infection can be counteracted with prophylactic antivirals, it leads to other infections. In general, viral infections promote tumorigenesis through the disruption of apoptotic machinery of the infected cell, promoting escape of host immunesurveillance. Molecular mechanisms associated with viral infection-related malignancies in SOT are depicted in Figure 4.

Epstein- Barr Virus (EBV): EBV infection poses a 20- fold increased risk for post-transplant lymphoproliferative disorder (PTLD), including NHL and Hodgkin's lymphoma². Latent infection of EBV immortalizes B cells. Further, lack of cytotoxic T cells, resulting from T-cell depleting induction therapy for the treatment of acute graft rejection, fuels uncontrolled growth of EBV-infected B cells. Reports suggest that EBV infection results in the production of abnormally high levels of IL-6, leading to growth of B cells¹. Thus, IL-6 plays a critical role in the pathogenesis of PTLD. In addition, EBV infection induced secretion of IL-10, a cytokine with immunosuppressive properties, aids in tumor development¹⁷.

Human herpes virus (HHV): HHV8 infection has been associated with increased risk of Kaposi's sarcoma (KS) after SOT with an SIR of 64.4⁵. HHV8-encoded viral Fas-associated death domain-like interleukin-1 β -converting enzyme-inhibitory protein (vFLIP) suppresses CD95 (APO-1/Fas)-mediated apoptosis. Binding of CD95L to CD95 and association with Fas-associated Death-Domain containing protein (FADD) leads to the formation of death-inducing signaling complex (DISC), which activates procaspase-8 mediated apoptosis. However, recruitment of cFLIP (cellular FLIP) at DISC inhibits procaspase-8 activation. vFLIP, comparable to cFLIP, disrupts DISC activity and inhibits apoptosis. CD95-mediated apoptosis is essential for immune-mediated elimination of viral infected cells. Therefore, vFLIP expression helps HHV8 infected cells evade host immunosurveillance¹⁸.

The B-cell lymphoma 2 (Bcl-2) family of proteins regulate both pro- and anti-apoptotic pathways. Bcl-2 inhibits apoptosis and promotes cell survival. HHV-8-encoded viral G protein-coupled receptor (vGPCR) increases the expression of Bcl-2 and inhibits apoptosis, leading to uncontrolled proliferation of the virus-infected cells¹⁹. Additionally, vGPCR is pro-angiogenic and increases the expression of many angiogenic factors including VEGF and angiopoietin-2 as well as angiogenic receptors including VEGF and angiopoietin receptors²⁰. Endothelial cells play a major role in angiogenesis and reports suggest that vGPCR activates VEGF receptors (VEGFRs) and promotes the proliferation of endothelial cells²⁰. Remarkably, vFLIP also protects the endothelial cells from super-oxide-induced apoptosis through the expression of anti-oxidant and anti-apoptotic MnSOD¹⁸.

Human Papillomavirus (HPV): HPV-encoded E6 and E7 proteins inhibit p53-mediated apoptosis and promote malignant proliferation of host cells²¹.

Hepatitis B virus (HBV) and Hepatitis C Viruses (HCV): Reports suggest that HBV and HCV infections are risk factors in post-transplantation liver cancer. HBV-encoded HBx protein and HCV-encoded NS3 and NS5A proteins play a critical role in hepatic carcinogenic mechanisms. HBx promotes Ras activation; and both HBx and NS5A promote PI3K-Akt survival pathway. Interestingly, HBx and NS5A modulate RTK Epidermal Growth Factor Receptor (EGFR) expression and ligand binding activity, respectively, leading to enhancement of PI3K-Akt downstream signaling pathway. HBV infection leads to increased expression of VEGF and angiopoietin-1 and modulates liver angiogenesis. HBV and HCV viral proteins also modulate the transcriptional activity of proto-oncogenes c-Myc, c-Fos and c-Jun and promote cell proliferation²².

Immunosuppression-related impaired tumor immunosurveillance:

Tumor immunosurveillance is a complex interplay between tumor-intrinsic/derived factors and the ability of host immune system to recognize and eliminate tumor cells. When tumor cells "escape" the attack of immune cells, tumor development is favored. Besides promoting cell-intrinsic pro-tumorigenic pathways, immunosuppressive agents can also modulate host tumor immunosurveillance and contribute to post-transplantation malignancies.

In addition to inhibiting T cell functions, immunosuppression changes the phenotype of other immune cells. In transplant patients with cancer, CD8⁺ T cells have increased expression of CD57, a marker of T cells immunosenescence. T-bet is a key transcription

factor regulating cytotoxic CD8⁺ T cell functions. Interestingly, CNI treatment, but not mTOR inhibition, downregulated T-bet expression in CD8⁺ T cells and drastically affected cancer cytotoxicity *in vivo*²³. Regulatory T cells (T reg), a subset of T cells (CD4⁺FOXP3⁺CD127^{low}), suppress activation, proliferation and cytokine production of effector T cells. T reg cells are essential to check uncontrolled/excessive immune reactions and to maintain immune homeostasis. However, abnormal increase in T reg cells suppresses effector T cell functions and aids tumor cells escape immune-mediated attack. CNI use has been associated with higher levels of T reg cells in the peripheral blood of kidney transplant recipients¹. CNI treatment affects T-cell motility and migration of dendritic cells in response to chemokines *in vitro*²⁴. Natural Killer (NK) cells can recognize and eliminate tumor or virus infected cells through the release of cytotoxic granules. Decreased numbers of NK cells and reduced NK cell function are associated with cancers in organ transplant recipients¹. In addition, CNI-induced secretion of immunomodulatory factors, such as TGF- β and IL-6 (which regulate B cell activation and proliferation), and other host-derived immunosuppressive factors, such as IL-10 and adenosine, can negatively affect tumor immunosurveillance involving both innate and adaptive immunity system¹. Potential mechanisms associated with immunosuppression-related impaired tumor immunosurveillance are schematically shown in Figure 5.

References

1. Au E, Wong G, Chapman JR. Cancer in kidney transplant recipients. *Nat Rev Nephrol*. 2018.
2. Sprangers B, Nair V, Launay-Vacher V, Riella LV, Jhaveri KD. Risk factors associated with post-kidney transplant malignancies: an article from the Cancer-Kidney International Network. *Clin Kidney J*. 2018;11(3):315–329. [PubMed: 29942495]
3. van de Wetering J, Roodnat JJ, Hemke AC, Hoitsma AJ, Weimar W. Patient survival after the diagnosis of cancer in renal transplant recipients: a nested case-control study. *Transplantation*. 2010;90(12):1542–1546. [PubMed: 21076383]
4. Hall EC, Pfeiffer RM, Segev DL, Engels EA. Cumulative incidence of cancer after solid organ transplantation. *Cancer*. 2013;119(12):2300–2308. [PubMed: 23559438]
5. Engels EA, Pfeiffer RM, Fraumeni JF, Jr., et al. Spectrum of cancer risk among US solid organ transplant recipients. *JAMA*. 2011;306(17):1891–1901. [PubMed: 22045767]
6. Akhtar MZ, Sutherland AI, Huang H, Ploeg RJ, Pugh CW. The role of hypoxia-inducible factors in organ donation and transplantation: the current perspective and future opportunities. *Am J Transplant*. 2014;14(7):1481–1487. [PubMed: 24909061]
7. Balan M, Chakraborty S, Flynn E, Zurakowski D, Pal S. Honokiol inhibits c-Met-HO-1 tumor-promoting pathway and its cross-talk with calcineurin inhibitor-mediated renal cancer growth. *Sci Rep*. 2017;7(1):5900. [PubMed: 28724911]
8. Akhtar MZ, Henderson T, Sutherland A, Vogel T, Friend PJ. Novel approaches to preventing ischemia-reperfusion injury during liver transplantation. *Transplant Proc*. 2013;45(6):2083–2092. [PubMed: 23953517]
9. Basu A, Contreras AG, Datta D, et al. Overexpression of vascular endothelial growth factor and the development of post-transplantation cancer. *Cancer Res*. 2008;68(14):5689–5698. [PubMed: 18632621]
10. Jung JW, Overgaard NH, Burke MT, et al. Does the nature of residual immune function explain the differential risk of non-melanoma skin cancer development in immunosuppressed organ transplant recipients? *Int J Cancer*. 2016;138(2):281–292. [PubMed: 25612559]
11. Perez CP, Patel N, Mardis CR, Meadows HB, Taber DJ, Pilch NA. Belatacept in Solid Organ Transplant: Review of Current Literature Across Transplant Types. *Transplantation*. 2018.

12. Gaumann A, Schlitt HJ, Geissler EK. Immunosuppression and tumor development in organ transplant recipients: the emerging dualistic role of rapamycin. *Transpl Int.* 2008;21(3):207–217. [PubMed: 18069922]
13. Datta D, Contreras AG, Basu A, et al. Calcineurin inhibitors activate the proto-oncogene Ras and promote protumorigenic signals in renal cancer cells. *Cancer Res.* 2009;69(23):8902–8909. [PubMed: 19903851]
14. Datta D, Contreras AG, Grimm M, Waaga-Gasser AM, Briscoe DM, Pal S. Calcineurin inhibitors modulate CXCR3 splice variant expression and mediate renal cancer progression. *J Am Soc Nephrol.* 2008;19(12):2437–2446. [PubMed: 18832436]
15. Basu A, Banerjee P, Contreras AG, Flynn E, Pal S. Calcineurin inhibitor-induced and Ras-mediated overexpression of VEGF in renal cancer cells involves mTOR through the regulation of PRAS40. *PLoS One.* 2011;6(8):e23919. [PubMed: 21886838]
16. Hojo M, Morimoto T, Maluccio M, et al. Cyclosporine induces cancer progression by a cell-autonomous mechanism. *Nature.* 1999;397(6719):530–534. [PubMed: 10028970]
17. Martinez OM, de Grujil FR. Molecular and immunologic mechanisms of cancer pathogenesis in solid organ transplant recipients. *Am J Transplant.* 2008;8(11):2205–2211. [PubMed: 18801025]
18. Thureau M, Marquardt G, Gonin-Laurent N, et al. Viral inhibitor of apoptosis vFLIP/K13 protects endothelial cells against superoxide-induced cell death. *J Virol.* 2009;83(2):598–611. [PubMed: 18987137]
19. Abboud ER, Shelby BD, Angelova M, et al. Kaposi sarcoma-associated herpesvirus g protein-coupled receptor enhances endothelial cell survival in part by upregulation of bcl-2. *Ochsner J.* 2013;13(1):66–75. [PubMed: 23532945]
20. Jham BC, Montaner S. The Kaposi's sarcoma-associated herpesvirus G protein-coupled receptor: Lessons on dysregulated angiogenesis from a viral oncogene. *J Cell Biochem.* 2010; 110(1): 1–9. [PubMed: 20213674]
21. Tomaic V Functional Roles of E6 and E7 Oncoproteins in HPV-Induced Malignancies at Diverse Anatomical Sites. *Cancers (Basel).* 2016;8(10).
22. Zemel R, Issachar A, Tur-Kaspa R. The role of oncogenic viruses in the pathogenesis of hepatocellular carcinoma. *Clin Liver Dis.* 2011;15(2):261–279, vii–x. [PubMed: 21689612]
23. Rovira J, Renner P, Sabet-Baktach M, et al. Cyclosporine A Inhibits the T-bet-Dependent Antitumor Response of CD8(+) T Cells. *Am J Transplant.* 2016;16(4): 1139–1147. [PubMed: 26855194]
24. Datta A, David R, Glennie S, et al. Differential effects of immunosuppressive drugs on T-cell motility. *Am J Transplant.* 2006;6(12):2871–2883. [PubMed: 17061998]

Key points:

- Clinical data reveal that there is at least 2- fold overall increased risk of cancer among transplant recipients, compared with general population.
- Immunosuppression is essential to prevent graft rejection; however, it contributes to post-transplantation cancer development.
- Molecular mechanisms of immunosuppressive agent-induced cell-intrinsic tumorigenic pathways are summarized.
- The role(s) of oncogenic virus infection in promoting transplant related malignancies are discussed.
- Immunosuppression negatively impacts the host tumor immunosurveillance mechanisms, resulting in the unhindered growth of tumors.

SYNOPSIS

Immunosuppression is essential to prevent graft rejection. However, immunosuppression impairs the ability of the host immune system to control viral infection and reduces tumor immunosurveillance. Therefore, immunosuppression after organ transplantation is a major risk factor for post-transplantation cancer. Notably, recent reports suggest that immunosuppressive agents can activate tumorigenic pathways independent of the involvement of the host immune system. In this review, we focus on cell-intrinsic tumorigenic pathways directly activated by immunosuppressive agents and also discuss the much-described infection- and immune-mediated mechanisms of cancer development in organ transplant recipients.

FIGURE 1. Hypoxia-related signaling molecules in post-transplantation cancer.

Under normoxic conditions, VHL protein targets HIFs for degradation. However, during IRI, hypoxia and ROS generation activate HIFs. Activated HIFs translocate to nucleus and bind hypoxia response elements (HRE) in the promoter regions of hypoxia-inducible genes, such as HO-1 and MnSOD, and angiogenic factors (like VEGF), leading to overexpression of these molecules.

FIGURE 2. Molecular targets of immunosuppressive agents.

Top left: Antigen presentation via MHC on APCs and co-stimulatory interactions (like B7–1/CD28) activate TCR, and it leads to an increase in the intracellular calcium levels and activation of calmodulin (CaM), which in turn binds and activates calcineurin.

Phosphorylated NFAT is inactive and resides in the cytoplasm. Activated calcineurin, with its protein phosphatase activity, dephosphorylates NFAT and promotes the nuclear translocation of NFAT to activate transcription of NFAT target genes, including IL-2, IL-4 and IFN- γ , leading to T cell activation. Co-inhibitory interactions like B7–2/CTLA-4 suppress TCR-mediated signaling. Recombinant fusion CTLA4-Ig can disrupt B7–1/CD28 and other co-stimulatory signals necessary for T cell activation. CNIs form drug/FKBP (or cyclophilin) complex and inactivate calcineurin. *Right:* mTOR is a serine/threonine protein kinase and is one of the downstream effector molecules for IL-2/IL-2R (and other growth factors)-induced PI3K-Akt signaling pathways. PTEN inhibits Akt activation through PI3K

suppression. mTORC1, the functional protein complex containing RAPTOR, regulates protein translation by modulating the activity of p70S6K and eIF4E (through 4E-BP). mTOR inhibitors form drug/FKBP complex and inhibit mTOR. Anti-IL-2R antibody blocks the activation of IL-2R. *Bottom left:* MMF and MPA inhibit IMDH-dependent *de novo* synthesis of guanosine nucleoside. AZA and 6-MP antagonize endogenous purines and prevent the incorporation of guanosine into DNA.

FIGURE 3. Immunosuppressive agent-induced cell-intrinsic tumorigenic pathways.

Right: RTK c-Met-mediated signaling promotes the activation of Ras-Raf-MAPK pathways and the nuclear translocation of Nrf2, leading to overexpression of HO-1. CNI enhances c-Met-induced Ras activation and HO-1 expression. CNI inhibits RKIP and carabin, the negative regulators of Ras, and contributes to sustained Ras activation in cancer cells. CNI promotes the expression of ATF3, and it can inhibit p53-mediated apoptotic pathways.

Center: CNI increases the expression of VEGF via PI3K-Akt-mTORC1 pathway channeled through HIFs. CNI-induced Ras-Raf-PKC- ζ activation inhibits PRAS40, the negative regulator of mTORC1, and augments mTORC1 activity. CNI treatment increases the expression of TGF- β , IL-6, and CXCR3-A (the growth promoting CXCR3 isoform).

Left: IL-6-mediated signaling activates JAK-STAT3 pathway. Together, CNI-induced signaling activates cell-intrinsic pro-tumorigenic pathways.

FIGURE 4. Oncogenic viral infections in post-transplantation malignancies.

Top Left: Binding of CD95(Fas/APO-1)L to CD95(Fas/APO-1) initiates the formation of DISC involving pro-caspase-8 and FADD resulting in the activation of pro-caspase-8. Subsequent activation of other caspases promotes apoptosis. HHV-encoded vFLIP interferes with the activation of pro-caspase-8 at DISC and inhibits apoptosis. HHV-encoded vGPCR promotes Ras-PI3K-Akt survival pathway and increases the expression of anti-apoptotic Bcl-2. *Right:* HBV-encoded HBx and HCV-encoded NS3 and NS5A proteins activate Ras-PI3K-Akt survival pathway. HBx and NS5A modulate RTK EGFR-mediated signaling. HBx modulates the transcriptional activity of c-Myc, c-Fos and c-Jun and increases the expression of angiogenic factors, such as VEGF and angiopoietin-1. *Bottom:* HPV-encoded E6 and E7 proteins inhibit p53-mediated apoptosis. EBV infection increases the secretion of IL-6 and IL-10. Together, oncogenic virus infection disrupts the apoptotic machinery of infected cells and also promotes angiogenesis, resulting in the malignant transformation of the infected cell.

FIGURE 5. Immunosuppression-related impaired tumor immunosurveillance.

Important changes observed in the immune phenotype of transplant recipients, as a result of immunosuppression, are summarized. CNI regimen has been associated with an increase in T reg cells and increased production of IL-6 from B cells. Increase in immunosenescent T cells and impaired NK cell cytotoxicity were observed in organ transplant recipients treated with CNI. Also, CNI treatment downregulated T-bet expression in CD8⁺ T cells and drastically affected its cytotoxic effector functions *in vivo*. Taken together, immunosuppression alters the phenotype and functions of immune cells, leading to impaired tumor immunosurveillance.