Calcineurin in skin : rising star or fallen angel?

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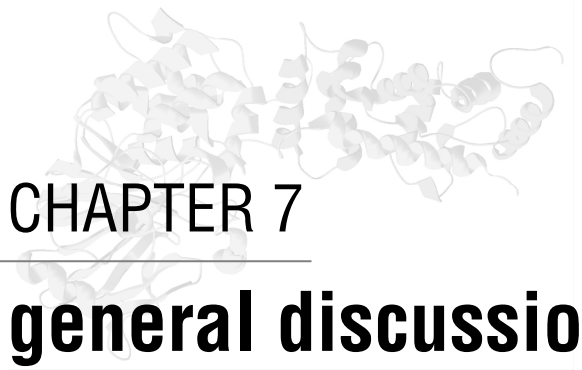

CHAPTER 7

general discussion and future prospects

The inclusion of calcineurin inhibitors such as cyclosporin A and tacrolimus in therapeutic regimens designed to prevent rejection in allograft recipients has contributed greatly to long-term survival after organ transplantation. However, one of the major long-term complications of these immunosuppressive protocols is the alarmingly increased incidence of skin cancer. The risk of skin cancer development seems to be proportional to the level of immunosuppression. In fact, a recent series of publications by Giese and Sommerer and coworkers illustrates that there is only a rather narrow window in which therapeutic Cn inhibition is sufficiently effective, yet the risk of skin cancer development is not significantly higher than normal (*1*, *2*). Therefore, individually tailored and thoroughly monitored immunosuppression seems to be of paramount importance in treatment protocols featuring calcineurin inhibitors.

An intriguing conundrum is the prevailing opinion that topical CnI, despite some controversy and a black box warning issued by the FDA, do not increase risk of skin cancer (*3-5*). This could be a dose issue, as systemically administered CnI can accumulate in skin and body fat, whereas topical CnI are usually applied locally and typically for a limited amount of time and could therefore undergo swift metabolisation and/or excretion, which essentially voids the risk of accumulation in other skin regions. Moreover, only very small amounts of topically applied CnI reach the bloodstream, resulting in very low systemic exposure (*6*).

UV radiation is the principal risk factor for skin cancer development; in addition to causing DNA damage, mutations and oxidative stress, it leads to both local and systemic immunodeficiency. The dependence of skin cancer incidence on CnI dosing and level of Cn signaling suggests a degree of interplay and maybe even synergism between Cn inhibition and UV radiation in the development of skin cancer that should be evaluated. This dissertation shows that UVA-1 radiation, in doses that could be obtained, for instance, by spending a day in the sun in Southern Europe, has the potential to negatively affect calcineurin activity in skin, supplemental to the effects of the CnI. This may represent an alternative explanation for the immunosuppressive features of UVA radiation. Photosensitization seems to be an important causal factor for the effects of UVA on Cn, involving both superoxide and singlet oxygen inflicting structural damage to the enzyme, which translates to diminished nuclear translocation of NFAT and decreased production of several pro-inflammatory cytokines (see chapters 3 and 4). The sensitivity of Cn to ROS also shows when cells are exposed to arsenite, which is thought to stimulate NADPH oxidase to produce large quantities of superoxide (chapter 5). Based on the known negative effects of UV radiation and CnI on Langerhans cell density and antigen presentation (*7-9*), a clearer dissection of the role of Cn signaling in Langerhans cells and the possible consequences of oxidative damage to Cn in relation to the efficacy and efficiency of tumor immunosurveillance in skin should be pursued.

Meanwhile, studies into the functions of Cn in skin cells have led to growing awareness of the importance of Cn signaling for a variety of cellular processes in

keratinocytes, melanocytes, and fibroblasts that are part of our tumor suppression armamentarium, including control of proliferation, cell cycle regulation, control of apoptosis, and DNA repair (*10-18*). ROS are capable of damaging DNA directly, but unfortunately also affect the fail-safe against this damage: a number of pathways that guarantee an adequate response to this damage (e.g. apoptosis, cell cycle arrest, DNA repair) to suppress tumor formation. Thus, inactivation of the cellular protection machinery by UVA could enhance the mutagenic effects of UV radiation. Interestingly, long-term exposure to either UVA or arsenic leads to malignant transformation of HaCaT keratinocytes attended by resistance to apoptosis (*19*, *20*). Oxidative stress can deactivate Cn directly or via induction of endogenous inhibitors (*21*). In transplant recipients treated with calcineurin inhibitors, excessive repression of Cn signaling – by either high concentrations of CnI or concerted action of UVA and CnI – could very well imply that Cn activity and Cn-dependent gene expression are pushed beyond "safe" limits.

Unfortunately, despite the increasing amount of cellular and molecular evidence suggesting involvement of Cn in skin carcinogenesis, almost no studies exist that establish a more definitive relation between Cn activity levels and clinical outcome. We propose a few lines of research to fill this gap.

First of all, it would be enlightening to verify whether the cumulative effect of UVA radiation and CnI presented in this dissertation is reflected at downstream levels as well, and whether the predictive value of the amount of NFAT-dependent gene expression in peripheral blood for cancer development, as described by Sommerer and Giese (*1*), can be extended towards Cn/NFAT signaling in skin cells. Ideally, it should be investigated how NFAT-dependent gene expression in peripheral blood correlates with CnI concentrations and markers of Cn activity in skin. Measurements of Cn activity and downstream markers such as NFAT in biopsies of UVA-irradiated and unexposed skin taken from transplant patients could deliver a fruitful contribution. Since the undertaking of such extensive studies is complicated by the invasive aspect of biopsy, the use of an engineered human skin model could be a viable alternative.

Secondly, a better specification of the exact role of components of the Cn signaling cascade in the etiology of tumor formation or facilitation of tumor growth should be pursued. Until recently, many experiments aimed to elucidate the link between Cn activity and procarcinogenic processes made use of CsA and/or TRL to modulate Cn activity. However, these CnI have secondary targets that have been related to tumor promotion (*22*, *23*) and may cloud the assessment of actual Cn involvement in the processes under investigation. In the field of DNA repair, following up on the original findings by Canning and Yarosh that CnI decrease nucleotide excision repair (NER) (*24*, *25*), Thoms *et al.* established a clean and direct link between Cn and NER using a Cn knockdown system (*18*). It would be interesting to further explore the course of events by which Cn knockdown or inhibition affects DNA repair. Such studies should help clarify which component of the DNA repair machinery is involved and whether this is a direct

effect of Cn or proceeds *via* NFAT. A recent publication suggests that Cn inhibition results in downregulation of xeroderma pigmentosum A and G genes, although the mechanism is still unclear (*26*). Even if Cn signaling is indeed mechanistically involved, there is still the fundamental issue with what activity range of Cn and/or NFAT these effects can be associated. This knowledge is important in light of the complex regulation of Cn activity (*27*), as illustrated, for instance, by the fact that the dose-response relationship between UVA-1 radiation (and other sources of oxidative stress) and Cn activity seems to exhibit a hormetic effect: while low doses stimulate Cn, effects of higher doses vary from declined stimulation to strong inhibition of Cn activity. Paradoxically, NFAT proteins are overexpressed in many tumors and envisaged to aid in tumor growth, survival and metastasis (*28-30*). Cn activity is also increased in several types of tumor cells, although reports of the opposite effect have appeared as well (*31, 32*). The dual roles of NFAT genes as oncogenes and tumor suppressors may take shape in different isoforms (*33, 34*).

It is evident that calcineurin is first and foremost an enzyme that is extremely sensitive to a multitude of external influences, particularly oxidative stress. Although it is plausible to assume that its redox sensitivity constitutes a basic regulation and adaptation mechanism, its universal involvement in Ca^{2+}-regulated processes in practically all our major organ systems combined with the high exposure to oxidative stress characteristic of the current Western lifestyle, makes this susceptibility a precarious issue and something that should be meticulously monitored. ROS may affect Cn activity in practically any cell and tissue type. The outcome, however, may differ fundamentally from one cell type to another, due to the variety in substrates, substrate kinetics, Cn isoforms, NFAT isoforms, co-transcripion factors and the gene selections under influence of NFAT.

The future of calcineurin inhibitors

Transplant patients taking CnI are nowadays advised to avoid sun exposure as much as possible. In response to the growing awareness of the risks of UVA, there is a trend among manufacturers of cosmetics to add antioxidants such as vitamins C and E and certain phenolic compounds c.q. flavonoids, mainly from plant origin, to sunscreen formulations (*35, 36*). In this way, not only is the amount of UV that reaches the skin reduced, but ROS generated by UV are neutralized as well. Additives that offer more specifically tailored prevention of Cn inhibition, adapted to the specific type of UV-induced damage (thiol compounds such as *N*-acetylcysteine being an obvious example (*37*)) may further improve the protection delivered by these suncreens and should be tested for efficacy against skin cancer development in transplant recipients. In addition, a new class of potentially viable alternatives to calcineurin inhibitors, mTOR inhibitors (sirolimus, everolimus), is emergent. mTOR inhibitors are characterized as antineoplastic (*38*) and do not display the severe nephrotoxicity seen with CsA and tacrolimus. Pioneering studies in which the use of CnI is completely abandoned, or studies in which

mTOR inhibitors are substituted for CnI before irreparable damage has befallen the kidneys have been undertaken, and show results that are undoubtedly very welcome to renal graft recipients in particular (*39-41*). This, however, does not alter the fact that the relation between Cn and skin cancer and other diseases is bound to remain a topic of broad interest. Cn inhibitors have at least one major advantage over mTOR inhibitors: a vast body of literature, consisting of extensive clinical as well as mechanistical and toxicological studies and reflecting over 30 years' experience, which gives a fair indication what to expect in the long run (*42*). While it does not seem that mTOR inhibitors show improved rejection rates compared to CnI (*43*), side-effects such as hepatotoxicity and blood disorders are already starting to surface (*44*), which is hardly surprising, as mTOR is not specific to immune cells any more than Cn. Some studies even conclude that the use of sirolimus for certain types of transplants should be discouraged (*45*), although these claims are disputed by others (*46*). Meanwhile, upgrades and improvements to the current arsenal of CnI, as illustrated by the development of voclosporin – a next-generation CnI that shows similar results to cyclosporine, yet is better tolerated (*47*) –, in combination with thorough individual monitoring in order to early diagnose adverse effects such as nephrotoxicity, could ensure that the moment to part ways with calcineurin inhibitors will not be anytime soon.

REFERENCES

1. Sommerer C, Hartschuh W, Enk A, Meuer S, Zeier M, Giese T. Pharmacodynamic immune monitoring of NFAT-regulated genes predicts skin cancer in elderly long-term renal transplant recipients. Clin Transplant 2008;22:549-54.
2. Giese T, Sommerer C, Zeier M, Meuer S. Monitoring immunosuppression with measures of NFAT decreases cancer incidence. Clin Immunol 2009;132:305-11.
3. Munzenberger PJ, Montejo JM. Safety of topical calcineurin inhibitors for the treatment of atopic dermatitis. Pharmacotherapy 2007;27:1020-8.
4. Ring J, Mohrenschlager M, Henkel V. The US FDA 'black box' warning for topical calcineurin inhibitors: an ongoing controversy. Drug Saf 2008;31:185-98.
5. Thaci D, Salgo R. Malignancy concerns of topical calcineurin inhibitors for atopic dermatitis: facts and controversies. Clin Dermatol 2010;28:52-6.
6. Breuer K, Werfel T, Kapp A. Safety and efficacy of topical calcineurin inhibitors in the treatment of childhood atopic dermatitis. Am J Clin Dermatol 2005;6:65-77.
7. Furue M, Katz SI. Cyclosporine A inhibits accessory cell and antigen-presenting cell functions of epidermal Langerhans cells. Transplant Proc 1988;20:87-91.
8. Al-Daraji WI, Grant KR, Ryan K, Saxton A, Reynolds NJ. Localization of calcineurin/NFAT in human skin and psoriasis and inhibition of calcineurin/NFAT activation in human keratinocytes by cyclosporin A. J Invest Dermatol 2002;118:779-88.
9. Seite S, Zucchi H, Moyal D, Tison S, Compan D, et al. Alterations in human epidermal Langerhans cells by ultraviolet radiation: quantitative and morphological study. Br J Dermatol 2003;148:291-9.

10. Wang HG, Pathan N, Ethell IM, Krajewski S, Yamaguchi Y, et al. Ca2+-induced apoptosis through calcineurin dephosphorylation of BAD. Science 1999;284:339.

11. Baksh S, DeCaprio JA, Burakoff SJ. Calcineurin regulation of the mammalian G0/G1 checkpoint element, cyclin dependent kinase 4. Oncogene 2000;19:2820-7.

12. Santini MP, Talora C, Seki T, Bolgan L, Dotto GP. Cross talk among calcineurin, Sp1/Sp3, and NFAT in control of p21(WAF1/CIP1) expression in keratinocyte differentiation. Proc Natl Acad Sci U S A 2001;98:9575-80.

13. Mammucari C, Tommasi di Vignano A, Sharov AA, Neilson J, Havrda MC, et al. Integration of Notch 1 and calcineurin/NFAT signaling pathways in keratinocyte growth and differentiation control. Dev Cell 2005;8:665-76.

14. Ryeom S, Baek KH, Rioth MJ, Lynch RC, Zaslavsky A, et al. Targeted deletion of the calcineurin inhibitor DSCR1 suppresses tumor growth. Cancer Cell 2008;13:420-31.

15. Krieg C, Boyman O. The role of chemokines in cancer immune surveillance by the adaptive immune system. Semin Cancer Biol 2009;19:76-83.

16. Wu X, Nguyen BC, Dziunycz P, Chang S, Brooks Y, et al. Opposing roles for calcineurin and ATF3 in squamous skin cancer. Nature 2010;465:368-72.

17. Dotto GP. Calcineurin signaling as a negative determinant of keratinocyte cancer stem cell potential and carcinogenesis. Cancer Res 2011;71:2029-33.

18. Thoms KM, Kuschal C, Oetjen E, Mori T, Kobayashi N, et al. Cyclosporin A, but not everolimus, inhibits DNA repair mediated by calcineurin: implications for tumorigenesis under immunosuppression. Exp Dermatol 2011;20:232-6.

19. Sun Y, Kojima C, Chignell C, Mason R, Waalkes MP. Arsenic transformation predisposes human skin keratinocytes to UV-induced DNA damage yet enhances their survival apparently by diminishing oxidant response. Toxicol Appl Pharmacol 2011;255:242-50.

20. He YY, Pi J, Huang JL, Diwan BA, Waalkes MP, Chignell CF. Chronic UVA irradiation of human HaCaT keratinocytes induces malignant transformation associated with acquired apoptotic resistance. Oncogene 2006;25:3680-8.

21. Lee JE, Jang H, Cho EJ, Youn HD. Down syndrome critical region 1 enhances the proteolytic cleavage of calcineurin. Exp Mol Med 2009;41:471-7.

22. Dantal J, Soulillou JP. Immunosuppressive drugs and the risk of cancer after organ transplantation. N Engl J Med 2005;352:1371-3.

23. Weischer M, Rocken M, Berneburg M. Calcineurin inhibitors and rapamycin: cancer protection or promotion? Exp Dermatol 2007;16:385-93.

24. Yarosh DB, Pena AV, Nay SL, Canning MT, Brown DA. Calcineurin inhibitors decrease DNA repair and apoptosis in human keratinocytes following ultraviolet B irradiation. J Invest Dermatol 2005;125:1020-5.

25. Canning MT, Nay SL, Pena AV, Yarosh DB. Calcineurin inhibitors reduce nuclear localization of transcription factor NFAT in UV-irradiated keratinocytes and reduce DNA repair. J Mol Histol 2006;37:285-91.

26. Kuschal C, Thoms KM, Boeckmann L, Laspe P, Apel A, Schon MP, Emmert S. Cyclosporin A inhibits nucleotide excision repair via downregulation of the xeroderma pigmentosum group A and G proteins, which is mediated by calcineurin inhibition. Exp Dermatol 2011.

27. Musson RE, Smit NP. Regulatory mechanisms of calcineurin phosphatase activity. Curr Med Chem 2011;18:301-15.

28. Jauliac S, Lopez-Rodriguez C, Shaw LM, Brown LF, Rao A, Toker A. The role of NFAT transcription factors in integrin-mediated carcinoma invasion. Nat Cell Biol 2002;4:540-4.

29. Buchholz M, Schatz A, Wagner M, Michl P, Linhart T, et al. Overexpression of c-myc in pancreatic cancer caused by ectopic activation of NFATc1 and the Ca2+/calcineurin signaling pathway. EMBO J 2006;25:3714-24.

30. Mancini M, Toker A. NFAT proteins: emerging roles in cancer progression. Nat Rev Cancer 2009;9:810-20.

31. Padma S, Sowjanya AP, Poli UR, Jain M, Rao B, Ramakrishna G. Downregulation of calcineurin activity in cervical carcinoma. Cancer Cell Int 2005;5:7.

32. Medyouf H, Alcalde H, Berthier C, Guillemin MC, dos Santos NR, et al. Targeting calcineurin activation as a therapeutic strategy for T-cell acute lymphoblastic leukemia. Nat Med 2007;13:736-41.

33. Robbs BK, Cruz AL, Werneck MB, Mognol GP, Viola JP. Dual roles for NFAT transcription factor genes as oncogenes and tumor suppressors. Mol Cell Biol 2008;28:7168-81.

34. Viola JP, Carvalho LD, Fonseca BP, Teixeira LK. NFAT transcription factors: from cell cycle to tumor development. Braz J Med Biol Res 2005;38:335-44.

35. Adhami VM, Syed DN, Khan N, Afaq F. Phytochemicals for prevention of solar ultraviolet radiation-induced damages. Photochem Photobiol 2008;84:489-500.

36. Nichols JA, Katiyar SK. Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. Arch Dermatol Res 2010;302:71-83.

37. Kang S, Chung JH, Lee JH, Fisher GJ, Wan YS, Duell EA, Voorhees JJ. Topical N-acetyl cysteine and genistein prevent ultraviolet-light-induced signaling that leads to photoaging in human skin in vivo. J Invest Dermatol 2003;120:835-41.

38. Faivre S, Kroemer G, Raymond E. Current development of mTOR inhibitors as anticancer agents. Nat Rev Drug Discov 2006;5:671-88.

39. Flechner SM, Kobashigawa J, Klintmalm G. Calcineurin inhibitor-sparing regimens in solid organ transplantation: focus on improving renal function and nephrotoxicity. Clin Transplant 2008;22:1-15.

40. Groetzner J, Kaczmarek I, Schulz U, Stegemann E, Kaiser K, et al. Mycophenolate and sirolimus as calcineurin inhibitor-free immunosuppression improves renal function better than calcineurin inhibitor-reduction in late cardiac transplant recipients with chronic renal failure. Transplantation 2009;87:726-33.

41. Budde K, Becker T, Arns W, Sommerer C, Reinke P, et al. Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. Lancet 2011;377:837-47.

42. Hocker B, Tonshoff B. Calcineurin inhibitor-free immunosuppression in pediatric renal transplantation: a viable option? Paediatr Drugs 2011;13:49-69.

43. Sharif A, Shabir S, Chand S, Cockwell P, Ball S, Borrows R. Meta-analysis of calcineurin-inhibitor-sparing regimens in kidney transplantation. J Am Soc Nephrol 2011;22:2107-18.

44. Panaro F, Piardi T, Gheza F, Ellero B, Audet M, et al. Causes of sirolimus discontinuation in 97 liver transplant recipients. Transplant Proc 2011;43:1128-31.

45. Massoud O, Wiesner RH. The use of Sirolimus should be restricted in liver transplantation. J Hepatol 2011.

46. Trotter JF, McKenna GJ. Sirolimus - It doesn't deserve its bad Rap(a). J Hepatol 2011.

47. Cooper JE, Wiseman AC. Novel immunosuppressive agents in kidney transplantation. Clin Nephrol 2010;73:333-43.