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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 metabolism 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-transcription 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 sunscreens 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


