Risk factors for non-melanoma skin cancer (NMSC)
1. Skin carcinoma progression analyzed by genome-wide gene expression profiling and mimicked by validated organotypic in vitro human skin cancer models; towards an integrative system-biology approach and an in vitro screening method for targeted skin cancer therapies.
2. Genetic and environmental risk factors for the development of skin cancer in organ-transplant recipients.

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Study 1:The ultimate goal is to abrogate or reduce the excessive burden of carcinomas in organ transplant patients. We aim to elucidate and establish consistent changes in genomic and expression (both mRNA and miRNA) profiles in the course of tumor...

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Skin neoplasms malignant and unspecified
Study type	Observational invasive

Summary

ID

NL-OMON30521

Source
ToetsingOnline

Brief title
Risk factors for non-melanoma skin cancer

Condition

- Skin neoplasms malignant and unspecified

Synonym
skin cancer, squamous cell carcinoma

Research involving
Human

Sponsors and support

Primary sponsor: Leids Universitair Medisch Centrum
Source(s) of monetary or material Support: Ministerie van OC&W,KWF: UL2007-3910: Local effects of immunosuppressants in the skin and impact on UV carcinogenesis: minimizing the skin cancer burden in organ transplant recipients;ZonMw projectnummer: 11.400.0079 Development of validated organotypic in vitro models leading to improved therapy of skin cancer.

Intervention

Keyword: risk factors, skin cancer

Outcome measures

Primary outcome

Study 1:

Firm establishment of consistent shifts in expression profiles of ensembles of genes and/or miRNAs related to certain signaling pathways will provide solid ground for applications for external funding. First, to expand the findings on tumor progression to the proteomic level and enzyme activities. Second, to further refine and explore the potential of newly developed bioinformatical

algorithms, e.g. to extract ensembles of activated transcription factors and responsive pathways. In addition, the validated organotypic in vitro human skin cancer models will be used for evaluation of novel therapeutic options, significantly reducing the use of animals for experimentation.

Study 2:

We will identify genes, which are associated with an increased risk of SCC and/or HPV infection. This will enable us to identify organ-transplant recipients with an increased risk of SCC and/or HPV infection. These data will also be integrated with the results from the genome wide analyses performed in study 1.

Secondary outcome

Not applicable.

Study description

Background summary

Skin carcinoma is the most common cancer in white populations. The clinical problem of cutaneous squamous cell carcinomas (SCC) is particularly dramatic among immunosuppressed patients, e.g. renal-transplant recipients. The most important risk factor for development of SCC in these patients is lifelong immunosuppression. In addition, environmental factors including sun (UV) exposure and human papilloma virus (HPV) infection, and genetic factors including fair skin and genetic polymorphisms are suspected contributors to development of the early stages of SCC. For a better understanding of skin cancer oncogenesis and, ultimately, to reduce the excessive burden of carcinomas in organ-transplant patients, we plan to initiate two studies.

The first study focuses on the understanding of the oncogenic alterations in

skin cells leading to preneoplastic lesions and tumors as well as on the establishment and validation of organotypic in vitro skin cultures mimicking the various tumor stages. A basic understanding of these pathogenic mechanisms is anticipated to open up the possibility of well-targeted therapies circumventing the immunocompromised state of the transplant patients. Skin tumorigenesis is a multistep process and many gene mutations and other genetic alterations have been reported. The immediate goal of this first study is to identify pathways (instead of individual genetic changes) that are consistently involved in the formation of SCCs and their precursor lesions, actinic keratoses (AK). In addition, we aim to develop robust and validated in vitro human skin models mimicking SCC in various stages of development, enabling (high-throughput) studies leading to molecular targets for clinical intervention. The analysis of skin tumor progression and corresponding genetic alterations brings together the research interests and expertise of four LUMC departments: Dermatology, Nephrology, Medical Microbiology and Toxicogenetics. The unique material consisting of SCC and AK from the same patient allows for genetic analysis and in vitro modeling of the process of skin tumorigenesis in isogenic backgrounds.

Secondly, since organ transplantation is complicated by a highly increased risk of skin cancer after the transplantation, we plan to initiate a case-control study to identify genetic and environmental risk factors for the development of skin cancer in organ-transplant recipients. In addition, we plan to focus on potential associations of genetic polymorphisms and HPV infection with skin cancer. We plan to focus on genotyping of important candidate genes for skin cancer so that we will be able to assign persons at risk for skin cancer already before organ transplantation.

Altogether, firm establishment of consistent shifts in expression profiles of ensembles of genes related to certain signaling pathways will provide insight in the multistep SCC carcinogenesis. In addition, validated organotypic in vitro human skin cancer models will be used for evaluation of novel therapeutic options, significantly reducing the use of animals for experimentation. Furthermore, we aim to identify genes, which are associated with an increased risk of skin cancer and/or HPV infection. This will enable us to identify organ-transplant recipients with an increased risk of skin cancer and/or HPV infection. Data of all studies will be integrated, so as to ultimately reduce the burden of skin cancer in organ-transplant recipients.

Study objective

Study 1:
The ultimate goal is to abrogate or reduce the excessive burden of carcinomas in organ transplant patients. We aim to elucidate and establish consistent changes in genomic and expression (both mRNA and miRNA) profiles in the course of tumor development from normal skin through AK to SCC. Such results may reveal consistent changes in signaling pathways and allow identification of

molecular targets for effective intervention. In addition, to address the clinical problem, we aim to develop robust and validated in vitro human skin models mimicking SCC in various stages of development, enabling (high-throughput) studies leading to novel opportunities for clinical intervention.

Innovative Aspects

The possibility to follow AK and SCC development in an isogenic background forms a robust approach for the identification of affected key pathways. It will generate superior data since filtering to reject inter-individual variability is not required and fewer patients will be needed to obtain statistically relevant data. Instead of zooming in on single genes, we will identify affected cellular pathways, which will provide an improved and more consistent data interpretation. In addition, analysis of differential miRNA expression allows for possible identification of miRNAs associated with skin tumorigenesis. By complementary analysis of chromosomal rearrangements (CGH array analyses, SNP arrays, PCC) and gene silencing (methylation array analyses) in fresh skin (tumor) material we will identify the molecular causes for gene expression modulation. The establishment of organotypic in vitro human skin models mimicking skin carcinoma in various stages allows for mutual confirmation of gene and miRNA expression data. In addition to the obvious clinical goal, we intend to introduce a methodically innovative and versatile approach to experimental in vitro modeling of human cancer, ultimately eradicating the need for animal models as surrogates.

Study 2:

The current molecular-epidemiological project will study the interaction between genetic and environmental risk factors for squamous-cell carcinoma (SCC) in organ-transplant recipients: (a) which associations can be found between genetic polymorphisms and SCC; (b) how do HPV infection and sun exposure modulate these associations and (c) which associations can be found between genetic polymorphisms and HPV infection.

Study design

Study 1:

Organ-transplant patients with skin carcinomas are regularly seen in the outpatient dermatological clinic and have entered a long-term monitoring programme at this clinic. Biopsies of normal unexposed skin (eg. upper inner arm), AK, and SCC from the same patient (isogenic) will be collected and either subjected directly to whole genome and proteome analysis or to establish skin carcinoma models which will be subsequently analyzed.

In first instance, we aim to include a cohort of 15 patients. On average, one

patient per week may be subjected to the removal of SCC and concurrent removal of two AKs and two biopsies from normal unexposed skin (inner arm). A central biopsy will be taken from both the SCCs and AKs before submitting the tumor to the LUMC Department of Pathology for proper diagnosis and creation of a uniform group of patients. Blood samples will be drawn as reference material for genetic analyses. Except for the fresh material required for premature chromosome condensation (PCC) analyses (see below), half of the biopsy material will be snap frozen and it is expected that the same material can be used for m(i)RNA microarrays, CGH and methylation analysis. The other half of the material will be used for establishment of the in vitro organotypic human skin carcinoma models. After the first phase, processing of patient material and interpretation of the results will be evaluated before a second cohort of 20 patients will enter this combined study.

Organotypic in vitro human skin carcinoma models

For the generation of skin carcinoma models we will use the skin explant technique developed in our laboratory. This technique, initially pioneered by Boxman et al. for growth of SCC cells, and later further optimized by El Ghalbzouri et al, allows the expansion of keratinocytes as well as an increased life span of the skin explant within a 3D culture model. Skin models generated from normal skin, AK and SCC will be sampled in time (e.g. 2 and 8 weeks) and the keratinocytes will be subjected to a whole genome and targeted proteome analyses. All data arising from these skin models will be compared to expression data obtained directly from the epidermal cells from skin biopsies of transplant patients. By combining these results we aim to deduce a (pre)malignant molecular signature of the different stages of skin cancer and determine whether it is conserved and maintained in time within the respective human skin carcinoma models. Moreover, this combined approach will deliver biological endpoints which might be potential targets for the development of new treatment strategies of these tumors.

Laboratory material processing for direct analysis

For direct analysis of gene expression, part of each half biopsy will be sectioned for (immuno) histochemical analyses to check for admixtures to the tumor cells of stroma and infiltrating lymphocytes. About 25-50 sections of 20 µm thickness will be cut and trimmed to remove surrounding dermal tissue. These sections should at least contain 70% tumor cells. Normal skin biopsies will be treated according to the split skin method to separate the epidermis from the dermis. These isolated epidermal sections will be used as reference material for the array analyses of AKs and SCCs.

RNA and DNA isolation

If a biopsy contains too much admixture of stroma or infiltrating cells, it will not be processed for subsequent extraction of m(i)RNA and DNA. Recently,

several commercially available kits have been introduced that allow simultaneous isolation of RNA and DNA from the same tissue section. This allows us to isolate sufficient amounts of high quality RNA and DNA for further analyses from biopsy material. However, if too many SCC biopsies have to be rejected because of admixtures, we will start microdissecting the material to purify the tumor material. Evidently, the RNA and DNA then needs to be amplified before further analyses.

Genome wide analysis of gene expression and genomic alterations

Genome-wide assessment of gene expression is carried out by m(i)RNA microarray analysis. A pair-wise comparison of obtained expression data will discriminate between biological (individual primary samples) and experimental (organotypic skin models) variation within the group of test samples (e.g. unexposed skin, AK or SCC) and statistically significant differences between groups. Results of interest will be verified using quantitative RT-PCR and in situ hybridization. The outcomes of microarray analysis will be correlated to other genome-wide analyses, including SNP arrays or CGH arrays to detect gene amplifications or losses, and CpG islands methylation arrays. The challenge with this wealth of data lies in the extraction of a coherent and robust model. The presently available techniques can readily be employed to analyze tumor progression, and outline characteristic changes that evolve in the transition from normal (naïve) skin to AK, and from AK to SCC, and possibly further to metastatic SCC.

Bio-informatical analyses

Computational analysis is essential to transform the masses of generated data into a mechanistic understanding of disease. We will use up to date approaches in microarray expression analysis that aim at uncovering the modular organization and function of transcriptional networks and responses in cancer instead of zooming in on changes of single genes.

Targeted proteome analysis

First, genes of interest selected on the basis of their altered gene expression pattern (e.g. signal transduction pathways/ oncogenic pathways) in (pre)malignant lesions will be analyzed by Western blot and immunohistochemical techniques (depending on antibody availability) in the HSE generated from normal skin, AK and carcinoma. This will provide insight as to whether transcriptional differences indeed result in differences in protein expression. Second, we will use commercially available antibodies against activated (phosphorylated) signaling proteins demonstrated to play a role in early stages of carcinogenesis of skin cells or other epithelial tumors (e.g. MAP-kinase or PI3K/Akt signaling). Third, unbiased expression analysis of the epidermal proteins of skin models will be performed using quantitative proteomics approaches, including nanoHPLC and 2D-PAGE followed by mass spectrometry as described. Preliminary studies show that the use of fluorescent dyes greatly

enhances the sensitivity of the 2D-approach and predicts that multiple samples (e.g. HSE generated from normal skin, AK and carcinoma or HSE generated from carcinoma and sampled in time) can be separately labeled, combined and subjected to a single analysis in a 2D run. This will demonstrate quantitative differences of proteins between the different stages of tumor development. Such differentially expressed proteins will be subjected to mass spectrometric analysis for identification as described and subsequently validated by immunohistochemistry and/or Western blotting.

Mutational and HPV analyses

Tumors will be screened for the presence of known oncogenic factors (e.g. P53 mutations) and HPV to be correlated to results from the gene profiling analyses.

Premature chromosome condensation (PCC)

Chemical induction of premature chromosome condensation (PCC) will be used to analyze the chromosomal constitution of SCCs and AKs. The protein phosphatase inhibitor calyculin A is capable to induce PCC in cells isolated from freshly dissected adenomatous polyps of a patient (as small as 2-4 mm2) with hereditary colorectal cancer (Bezrookove et al, 2003). In fresh tumor specimens, a regimen of 80 nM calyculin A for 75 min after only 2 days of culturing, resulted in a PCC index of 2-5%. pq-COBRA-FISH (Combined Binary Ratio labeling-fluorescence in situ hybridization) will be used for molecular karyotyping of numerical and structural chromosomal abnormalities.

Model validation and proof of principle

We aim to stimulate the identified carcinogenic events in the skin by engineering HSEs that harbor characteristics of (pre)malignant skin tumors. Since future (high throughput) screening for therapeutics requires production of in vitro models on demand, we will start with exploring whether storage and reseeding of keratinocytes obtained from growing skin explants is a feasible approach, providing that the molecular (pre)malignant signature is maintained in skin explants. Generated 3D models will be validated for the presence of the (pre)malignant characteristics using the m(i)RNA and protein markers identified above. Finally, as proof of principle, we aim to assess whether metabolic blockers known to be effective in suppressing skin cancer outgrowth in both mice and human, are also effective in the treatment of skin models with (pre)malignant phenotype. Effects of treatment will be determined by analysis of gene and protein expression profiles.

Study 2:

Organ-transplant patients with a high tumor burden have entered a long-term monitoring program run by Dr. Jan Nico Bouwes Bavinck at the Dermatological clinic of the LUMC. We would like to include 150 organ-transplant recipients

with a history of SCC and 250 recipients without a history of skin cancer, matched for age, sex and time period after transplantation. Questionnaires will be used to collect data about sun exposure, smoking, etc. Hair bulbs supposedly represent the reservoir of skin HPV types. HPV infection will be measured by the presence of high-risk HPV types in plucked eyebrow hairs. Blood will be collected for the isolation of genomic DNA for genetic typing. We already collected these materials from 966 immunocompetent persons. The latter group, consisting of 161 patients with SCC, 426 with basal cell carcinoma or malignant melanoma and 386 persons without any skin cancer will be used as a control group representative for the non-immunosuppressed population.

HPV and genetic analyses

The presence of HPV in the plucked eyebrow hairs of the patients will be determined by established PCR-based assays as well as antibodies against viral early and late proteins. Genetic analyses will start with validation by confirming the association between SCC and IL-10 polymorphisms, which are reported to be associated with SCC in transplant recipients. Next additional, cytokine polymorphisms will be characterized, e.g. TNF*, IL-1*, IL-2, IL-4, and IL-6. Time and money permitting, more candidate genes (XP, EVER, GST, etc.) will be tested. Genotyping will be performed using the Sequenom MassArray system, a well-established platform for high-throughput genotyping using MALDI-TOF mass spectrometry that is present in the laboratory of department of Molecular Epidemiology of our Centre. The association between the genetic polymorphisms and SCC and the modulation of this association by HPV infection will be statistically analyzed by multivariate techniques.

Study burden and risks

Not applicable.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Organ-transplant patients and immunocompetent persons with and without skin cancer.

Exclusion criteria

No consent of the patient.

Study design

Design

Study type:	Observational invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	01-07-2007
Enrollment:	1500
Type:	Actual

Ethics review

Approved WMO

Application type:	First submission
Review commission:	METC Leiden-Den Haag-Delft (Leiden) metc-ldd@lumc.nl

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL14079.058.07