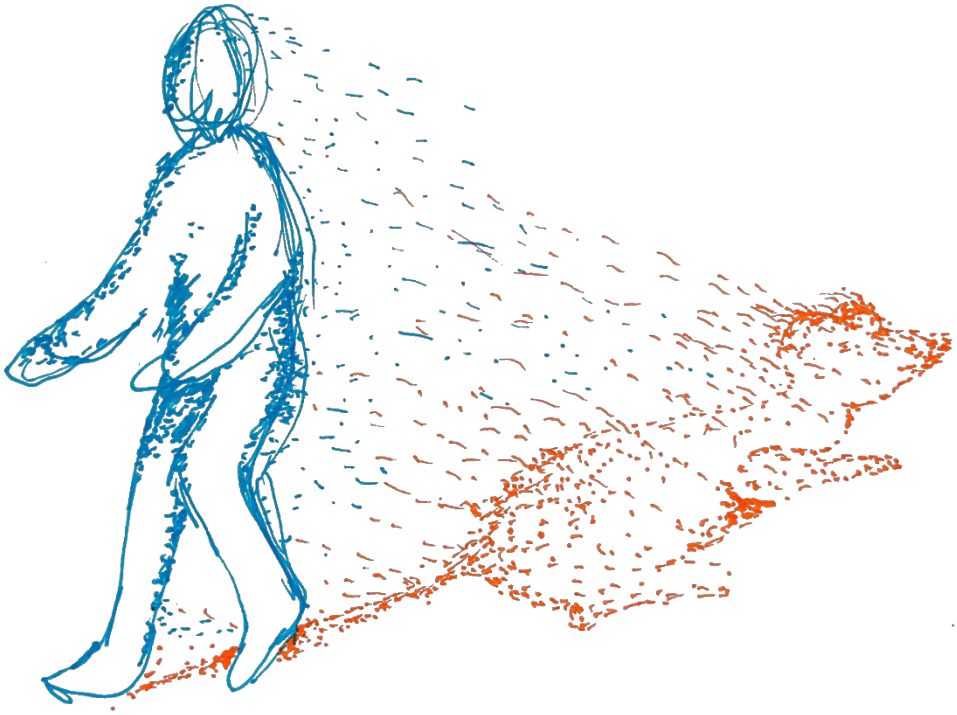
DTU Health Tech
Department of Health Technology

Danish Research Center
For Skin Cancer

Exploring Keratinocyte Carcinoma Models for Preclinical Treatment Evaluation

PhD thesis



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Abstract

Keratinocyte carcinoma arises in the keratinocytes of the skin and encompasses basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (cSCC). Keratinocyte carcinoma is the most common type of cancer and presents a major health and economical challenge for healthcare systems in the Western World. Current treatment options for inoperable cases of keratinocyte carcinoma are limited. In recent years, the approval of immune checkpoint inhibitors has marked a breakthrough in the treatment of keratinocyte carcinoma. This success has sparked renewed interest in developing new immunotherapies for keratinocyte carcinoma.

Mouse models play an irreplaceable role in the development of new treatments by providing a platform for evaluating therapeutic strategies and understanding treatment mechanisms. Despite the high prevalence of keratinocyte carcinoma, current mouse models of keratinocyte carcinoma are sparse and impractical to include in treatment evaluation studies due to their complexity or requirement of immunocompromised mice. This thesis presents the development and utilization of immunocompetent transplantable mouse models of keratinocyte carcinoma that mimic the current models but offer more practical models in preclinical drug development.

A transplantable model of BCC was established by serial passaging tumor cells *in vivo* from a well-established genetically engineered mouse model of BCC. Comparative analysis using histology, flow cytometry and RNA sequencing revealed that the transplantable BCC model resembled microscopic structures, immune cell populations and key transcriptional features of its parental genetically modified BCC model, while being more practical and resource efficient.

Building upon the same approach, a transplantable cSCC tumor model was established from an ultraviolet radiation-induced cSCC model. This cSCC model was utilized to evaluate the therapeutic potential of intratumoral resiquimod-gel (RSQ-gel), a toll-like receptor 7/8 agonist formulated into a sustained-release platform. The efficacy of the RSQ-gel was further compared with the clinically approved topical imiquimod cream treatment. Both treatments were evaluated alone and in combination with ablative fractional laser (AFL), a treatment previously shown to induce antitumor immune responses in preclinical studies. RSQ-gel treatment with adjuvant AFL demonstrated significant antitumor efficacy in the cSCC model. Additionally, the efficacy of weekly RSQ-gel was comparable to daily imiquimod treatment. This study highlights the utility of the transplantable cSCC model for initial preclinical evaluation of treatments for keratinocyte carcinoma.

AFL has been shown to induce both pro-inflammatory, antitumor immune responses in tumor models and anti-inflammatory, healing responses in skin tissue. To uncover the ambiguity of AFL immunological responses, AFL's impact on macrophages was investigated as they play a key role in both pro-inflammatory anticancer responses and anti-inflammatory, healing responses. AFL treatment of healthy mouse skin polarized macrophages towards an anti-inflammatory wound-healing-like phenotype characterized by an upregulation of arginase-1 protein. These findings suggest that AFL treatment potentially induces anti-inflammatory response that could counteract the immune activation required for effective antitumor immune responses.

In summary, this thesis presents the development of transplantable keratinocyte carcinoma models that provide practical and representative platforms for preclinical development. The practical application of the developed transplantable keratinocyte carcinoma models was demonstrated in the included preclinical evaluation study. This thesis also discovered that anti-inflammatory responses that resemble wound-healing processes occur in skin in response AFL treatment.

Dansk Resumé

Keratinocytkarcinom opstår i hudens keratinocytter og omfatter basalcellecarcinom (BCC) og kutant pladecellekarcinom (cSCC) Keratinocytkarcinom er den mest almindelige kræftform og udgør en stor sundhed og økonomisk udfordring for sundhedssystemerne i den vestlige verden. Nuværende behandlingsmuligheder for uoperable tilfælde af keratinocytkarcinom er begrænsede. I de senere år har godkendelsen af immuncheckpoint-hæmmere markeret et gennembrud i behandlingen af keratinocytkarcinomer. Denne succes har skabt fornyet interesse i at udvikle nye immunterapier mod keratinocytkarcinomer.

Musemodeller er uerstattelig under udviklingen af nye behandlinger. Modellerne muliggør evaluering af terapeutiske behandlinger og undersøgelsen af potentialle virkningssmekanismer. På trods af den høje forekomst af keratinocytkarcinomer, er de nuværende musemodeller af keratinocytkarcinom sparsomme og upraktiske at inkludere i behandlingsstudier på grund af deres kompleksitet eller behovet for immunkompromitterede mus. Denne afhandling præsenterer udviklingen og anvendelsen af immunkompetente transplantable musemodeller af keratinocytkarcinomer, der efterligner de nuværende modeller, men som er mere praktiske at inkludere i præklinisk lægemiddeludvikling.

En transplantabel model af BCC blev etableret ved serielt at passere tumorceller *in vivo* fra en veletableret genetisk modificeret musemodel af BCC. Komparativ analyse ved hjælp af histologi, flow cytometri og RNA-sekventering viste, at den transplantable BCC-model efterlignede de mikroskopiske strukturer, immuncellepopulationer og transkriptionelle træk fra sin genetisk modificerede forældre BCC-model, samtidig med at den var mere praktisk og ressourceeffektiv.

Med udgangspunkt i samme tilgang blev en transplantabel cSCC-tumormodel etableret fra en ultraviolet strålingsinduceret cSCC-model. Denne cSCC-model blev anvendt til at evaluere det terapeutiske potentiale af intratumoral resiquimod-gel (RSQ-gel), en toll-like receptor 7/8-agonist formuleret i en leveringsform der frigiver stoffet over tid. Effektiviteten af RSQ-gel blev yderligere sammenlignet med den klinisk godkendte topiske imiquimod-creme behandling. Begge behandlinger blev evalueret alene og i kombination med ablativ fraktioneret laser (AFL), en behandling, der tidligere har vist sig at inducere immunmedierede antitumor effekter i prækliniske studier. RSQ-gel-behandlinger med adjuverende AFL viste betydelig antitumor-effektivitet i cSCC-modellen. Desuden var effektiviteten af ugentlig RSQ-gel sammenlignelig med daglig imiquimod-behandling. Dette studie fremhæver anvendeligheden

af den transplantable cSCC-model til indledende præklinisk evaluering af behandlinger for keratinocytkarcinomer.

AFL har vist sig at inducere både pro-inflammatoriske antitumor immunmedierede reaktioner og antiinflammatoriske, helingsprocesser i huden. For at afdække tvetydigheden af AFL's immunologiske responser blev AFL's indvirkning på makrofager undersøgt, da de spiller en vigtig rolle i både pro-inflammatoriske, anticancer reaktioner og anti-inflammatoriske, helende reaktioner. AFL-behandling af rask mussehud polariserede makrofager mod en antiinflammatorisk sårhelende-fænotype, der er karakteriseret ved en opregulering af arginase-1 protein. Disse resultater tyder på, at AFL-behandling potentielt inducerer en antiinflammatorisk reaktion, der kan modvirke den immunaktivering, der kræves for effektive immunmedierede antitumor reaktioner.

Denne afhandling præsenterer udviklingen af transplantable keratinocytkarcinomer modeller, der fungerer som praktiske og repræsentative modeller i præklinisk udvikling. Den praktiske anvendelse af de udviklede transplantable keratinocytkarcinommodeller blev demonstreret i den efterfølgende prækliniske behandlingsevaluering. Denne afhandling fandt også, at antiinflammatoriske responser, der ligner sårhelingsprocesser, opstår i huden som reaktion på AFL-behandling.