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Dynamic control of Thyroid Hormone signaling regulates the progression of cutaneous Squamous Cell Carcinomas

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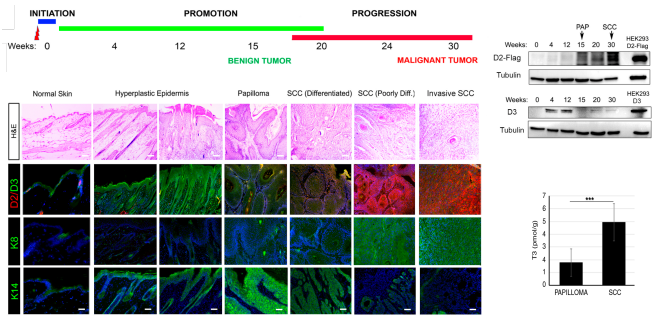
ABSTRACT

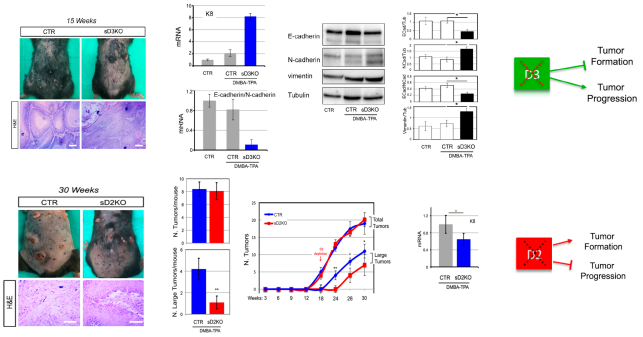
Non-melanoma skin cancer (NMSC) is the most common cancer in humans and include both, Basal Cell Carcinomas (BCCs) and Squamous Cell Carcinomas (SCCs). BCC is highly proliferative but rarely metastatic, while cutaneous squamous cell carcinoma (cSCC) can metastasize primarily to lymph nodes and then to liver and lungs, and accounts for ~20% of annual skin cancer-associated mortalities. Thyroid hormones (TH) T4 and T3 regulate the metabolism and growth of all cell types, and thereby have a strong impact on cancer. Indeed, from development to adult life, TH signaling regulates cell-fate determination and differentiation in normal and pathological contexts. Intracellular TH signaling is regulated within target cells via the action of the deiodinases D2 and D3, which catalyze TH activation and catabolism. In detail, D2 catalyses the activation of thyroid hormone by promoting the conversion of T4 into T3, while the inactivation of T4 and T3 into inactive metabolites occurs via inner ring deiodination mediated by D3. Hormonal regulation of tumorigenesis has often a critical role in cancer formation and their progression to malignancy. However, how TH impacts on the progression, invasiveness and metastasis of skin cancers is largely unknown. The aim of this study is to evaluate the involvement of TH in the progression of the NMSC and determine the effects of TH signaling and its regulators in the late stages of the neoplastic process. In order to investigate the role of TH and its metabolism in the progression, invasiveness and metastasis of skin cancers, we studied the expression of deiodinases D2 and D3 through in vitro experiments in deiodinases-depleted SCC cells by CRISPR/Cas9 technology and in vivo analysis on mouse models of epidermis specific deiodinases-depletion, subjected to two-step chemically-induced skin carcinogenesis. Taken together, our in vitro and in vivo experiments revealed that the effects of D2 and D3, and consequently, TH action, are uncoupled to the various phases of cSCC tumorigenesis. Indeed, while TH initially reduces tumor formation, it subsequently accelerates invasiveness and metastatic conversion.

D2 and D3 deiodinases are dynamically expressed during SCC tumor initiation and progression.



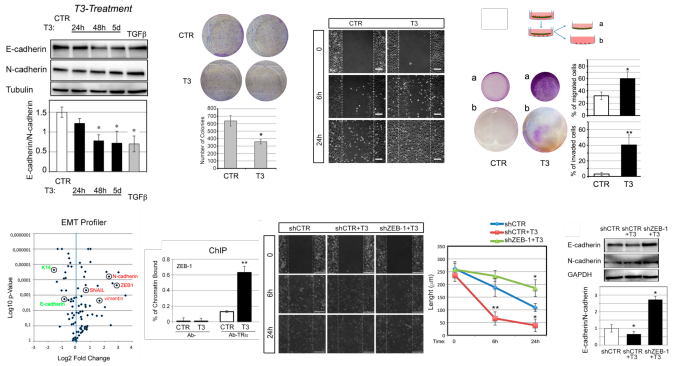
To investigate the role of TH and its metabolism in the progression, invasiveness and metastasis of skin cancers, we studied the expression of deiodinases D2 and D3 in different stages of SCC using the two-step chemically-induced carcinogenesis model. Immunofluorescence demonstrated that D3 rapidly increased during the initial tumorigenic step and peaked at the hyperplastic epidermis stage. Conversely, D2 expression began at later time points, reaching a nadir at the final phases of tumorigenesis when papillomas lose their differentiation potential, become more invasive and turn into SCCs. Western blot analysis confirmed the sequential expression of D3 and D2 at 15 and 30 weeks after DMBA/TPA treatment. Notably, the dynamic expression of D2 and D3 is consistent with lower intra-tumoral T3 levels in the papillomas then in the more advanced SCCs.

Thyroid hormone action in epidermal specific D3KO and D2KO mice.



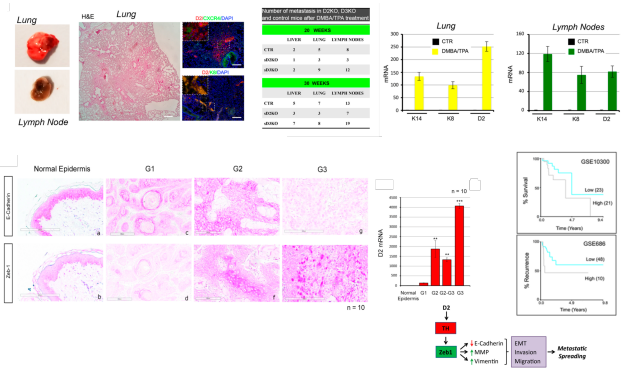
To gain insight into the role of TH in tumor progression, we disrupted the TH signal balance by depleting D2 or D3 in the epidermal compartment. First, we depleted D3, 15 weeks after treatment. The analysis of K14 and K6, showed that tumor formation was much lower in sD3KO mice than in CTR. D3-depletion reduced the frequency and the incidence of cancers. K8 expression was higher in D3KO mice than in CTR and the E-cadherin/N-cadherin ratio was lower in D3KO lesions than in control lesions, suggests that D3-depletion accelerates SCC formation. D2-depletion increased the frequency of hyperplastic lesions and papillomas and increased the level of K6 in D2KO papillomas. Notably, the greater tumor growth in D2KO mice was not associated with greater tumor progression and invasive conversion. Indeed, K8 expression was lower in sD2KO mice than in CTR mice thus indicating that D2KO papillomas resist progression to SCC.

Thyroid hormone activation induces the EMT, migration and invasion ability of SCC cells, by inducing ZEB-1 transcription.



We evaluated the contribution of the TH signal to the promotion of EMT in the SCC 13 cells. T3 treatment increased N-cadherin expression and slightly reduced E-cadherin expression, indicating increased EMT. Notably, we observed that T3 reduced proliferation but contextually, it enhanced the migration and the invasiveness of SCC cells. We next investigated the molecular mechanisms by which T3 induces EMT and invasiveness of SCC cells. We profiled the expression of 84 genes whose expression is related to EMT using the human EMT RT² Profiler™ PCR Array. To identify the EMT genes that mediate TH-dependent invasiveness, we first analyzed a ChIPseq of THR-binding sites. Among these T3-binding sites, T3 physically binds the ZEB-1 genes in SCC cells .To assess whether ZEB-1 is the molecular determinant of the T3-dependent EMT, ZEB-1 was down-regulated in SCC cells. ZEB-1 depletion completely rescued T3-dependent enhanced migration. mRNA and western blot analysis of the EMT genes confirmed that ZEB-1 depletion blocks the T3-dependent EMT cascade

D2 is Expressed in Tumor Metastases from SCC and is a Prognostic Marker of SCC Progression in Humans.



We evaluated whether D2 is expressed in metastatic lesions at distant sites. Metastatic lesions in the lung of D2-3xFlag tumorigenic mice were positive for D2, compared to non- tumorigenic mice, as demonstrated by double staining of D2/CXCR-4 and D2/K8. Accordingly, PCR analysis revealed that lungs and lymph nodes with micro-metastasis were highly positive for D2 mRNA and for K14 and K8 mRNA whereas these markers were barely detectable in control mice. We next evaluated the correlation of D2 expression in human tumors with the markers of EMT E-cadherin and ZEB-1, and the relative switch from papillomas to SCC. We collected 72samples of human tumors at different pathologic states and tumor grade up to cSCC, to assess the clinical significance of D2 and the TH signal in human SCC. We then measured the D2 mRNA expression from the same biopsies (n=10) and found that D2 mRNA was potently overexpressed in cSCC compared to normal epidermis, and that the highest fold change occurred in poorly differentiated cSCC. We also analyzed two collections of tumors in which the gene expression signature of SCC was associated to the risk of relapse, recurrence-free survival and overall survival of patients. Kaplan–Meier plots from both data sets showed a striking significant correlation between high D2 levels and risk of relapse, and an inverse correlation with the percent survival of patients.These results suggest that D2 levels are correlated with a more advanced tumor stage and with a poorer prognosis of human cancer.

In conclusion, in this study, we addressed the central question of how TH influences different phases of tumorigenesis By altering the TH signal via cell-specific deiodinase knock-down, we demonstrate that modulation of TH concentration can delay tumor cell growth and invasion depending on tumor stage, and can affect the malignant epithelial tumor phenotype. Therefore, we conclude that D2 is an endogenous ''metastasis promoter'' and that D2-inhibition can help to reduce human cancer metastasis. These findings provides the rationale for the concept that pharmacologically-induced TH inactivation could be a strategy to attenuate metastatic formations.