#3

BIOCHEMICAL CHANGES IN NORMAL SKIN CAUSED BY SQUAMOUS CELL CARCINOMA USING FTIR SPECTROSCOPY

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Background: Squamous cell carcinoma is the second most common skin cancer. The diagnosis requires biopsy followed by histopathological analysis, which is the gold standard diagnosis. The histopathology has some subjectivity and an auxiliary cancer detection method is necessary to accurately determine the presence of cancer cells still in early stages. Due to its molecular-level information, the FTIR technique has great potential to differentiate neoplastic from normal cells, making it a possible and powerful diagnostic tool.

Study: We used the FTIR spectroscopy and histopathological technique to analyse the biochemical and morphological changes in normal skin during the evolution of squamous cell carcinoma in 50 Swiss mice submitted to chemical carcinogenesis. Infrared spectra data of FFPP (Formalin-fixed paraffin-processed) sections of normal and tumoral skin were obtained with a Thermo Nicolet 6700 Fourier transform infrared spectrometer equipped with an attenuated total reflection (ATR) accessory. Hierarchical Cluster analysis (HCA) was used as an unsupervised classification technique in order to evaluate the similarity level between spectral data.

Results: Neoplastic lesions shown an intense proliferation of keratinocytes in an exophytic pattern. The basal layer epithelium displays a moderate dysplasia and hyperchromatism. Hyperkeratosis and papillary projections were frequently observed and some of lesions exhibited invasion of the neoplastic cells into the dermis, which showed intense collagen deposition and numerous blood vessels, which clearly indicates SCC. FTIR spectroscopy shown an increase in vibrational modes associated with RNA, suggesting an increased amount of this nucleic acid, that is consistent with increased protein bands intensity (Amide I and II).The decrease in intensity of collagen bands were observed and it is associated to potentially malignant carcinomas.

Conclusion: We have shown that FTIR spectroscopy was able to distinguish normal skin from cutaneous SCC and it is a promising auxiliary method during the diagnosis process.

#4

NON-INVASIVE DIAGNOSIS OF HEMANGIOMAS USING DOPPLER OCT IMAGING

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Background: Hemangiomas are vascular tumors of the dermis capillaries that often involve the head, neck and oral cavity, with light to severe disfigurement. Early diagnosis and recognition of the lesion is essential to provide appropriate treatment and decrease harmful cosmetic and psychological effects. Critical parameters for diagnosis are the presence of caverns, the density and diameter of blood vessels as well as the intensity of their blood flow. Excisional biopsies are the gold-standard but may cause severe bleedings and side-effects, so that a non-invasive imaging technique should be preferred.

Study: We propose to use Optical Coherence Tomography (OCT), a morphological *in vivo* optical imaging technique, coupled to functional blood-flow Doppler modality. We imaged hemangiomas on 14 child patients of the IMIP hospital. The OCT system is a Thorlabs swept source OCT at a wavelength of 1325 nm. The system has a frame rate of 25 images per second, axial and transverse resolution in tissue of 9 μm and 18 μm respectively, maximum detection depth in skin of approximately 1 mm.

Results: We were able to distinguish between normal skin and vascular lesion areas. The lesions present blood vessels of mean diameter 114 μm and mean depth 304 μm. Although quantitative value of blood flow was not assessed, we can qualitatively report a high variability of blood flow intensities.

Conclusion: Our findings in terms of density, size and depths of blood vessels are consistent with previous literature on the subject. The high standard-deviation of our statistics can be explained by the high variability of the lesions as well as variability of vascularity inside one lesion. We are currently enrolling more patients in the study to reach a total of 50 patients and increase statistical significance. We have shown that OCT completed by Doppler OCT is a promising method for non-invasive diagnosis and monitoring of vascular lesions.

#5

LIDOCAINE-INDUCED POTENTIATION OF THERMAL DAMAGE IN KERATINOCYTES, FIBROBLASTS, AND BASAL CELL CARCINOMA IN CULTURE

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Background: Lidocaine acts as a local anesthetic by blocking transmembrane sodium channel permeability, thereby disabling depolarization of neurons and inhibiting painful sensation. Lidocaine also induces the synthesis of heat shock proteins, sensitizes cells to hyperthermia and increases the aggregation of nuclear proteins during heat shock. We previously reported two cases of mature surgical scars treated with non-ablative fractional resurfacing, which developed deep focal ulceration at points corresponding to local lidocaine anesthetic injection sites, despite conservative settings, adequate time and cooling between passes. It was hypothesized that lidocaine had focally sensitized keratinocytes to the thermal damage of laser treatment. The goal of this study was to investigate the effect of lidocaine on heat sensation by using an in vitro model with cell lines representing the skin.

Study: We used human keratinocyte and fibroblast as well as murine basal cell carcinoma cell lines. Cells were seeded in multiwell plates and pre-incubated with lidocaine 1 hour prior heating. Cell viability was assessed 24 hour later by using the MTT assay.

Results: The results of this study show that lidocaine causes dose-dependent thermal hypersensitivity of epidermal and dermal cells. In cultured human keratinocytes and fibroblasts, survival at 44 C was significantly reduced by incubation with 0.1 and 0.2% lidocaine, concentrations much lower than what is used clinically when treating patients (1–2%). In a cultured murine basal cell carcinoma cell line, lidocaine caused even greater