superoxide (O_2^-). As the mitochondrial electron transport chain (mETC) is the major cellular generator of ROS, as a result of leakage of single electrons which reduce O_2 to O_2^-, it is desirable to monitor ROS generation from the mitochondria exclusively. We report a novel application of the amperometric sensor to monitor O_2^- generation in isolated mitochondrial fractions, directly and in real time. This is the first time that this type of measurement has been demonstrated and it allows the dynamics of mitochondrial O_2^- release following inhibition of complexes I (rotenone) and III (antimycin) of the mETC to be examined. We have also used mitochondrial DNA (mtDNA) as a damage marker to relate the real-time measurement of oxidative stress in cells to downstream DNA damage, using UVA and H_2O_2 as stressors. For this investigation we have employed a quantitative polymerase chain reaction assay to measure mtDNA strand breaks in pigmented and nonpigmented melanoma cells, HaCaTs and human dermal fibroblasts.

P59
Identification of therapeutic and prognostic biomarkers in keratinocyte carcinogenesis

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Cutaneous squamous cell carcinoma (SCC) is the second most common malignancy in humans which, along with its precursor lesion, keratinocyte intraepithelial neoplasia (KIN), results in substantial morbidity and burden on resources. Early identification and treatment of KIN is likely to have a significant impact on stemming the increasing burden of SCC. We have adopted a systematic integrated approach using genome-wide single nucleotide polymorphism (SNP) and expression microarrays to investigate the molecular basis of KIN by comparing genomic profiles of sequential stages of KIN evolution from normal nonsun-exposed (NSE), normal sun-exposed (SE) and KIN skin in paired skin biopsies from 31 immunosuppressed and 22 immunocompetent individuals. Nine series were excluded for clinical and technical reasons. SNP profiling compared with paired venous blood revealed twice as many genetic aberrations in KIN (305) compared with NSE (157) or SE (153) skin. The most common changes in KIN were amplification at 9p (18% of samples), 8q (14%) and 14q (11%) and loss at 9p (16%) and X (11%). Many regions were common to those seen in cutaneous SCC, such as 3p and 9p loss and gain at 8q. Results were stratified according to immune status and histological grade of KIN. Expression profiling compared with paired NSE skin revealed increasing gene dysregulation through the sequential stages of KIN and altered gene ontology biological processes in KIN towards metabolic processes and cell component biogenesis. Integration of genetic and expression data has revealed concordant areas of change where genetic events result in altered transcription and disconcordant expression changes where epigenetic mechanisms may be responsible. In summary, this study has explored key cellular events in KIN, which may identify important prognostic and therapeutic biomarkers for this common and rapidly increasing cancer.

P60
Assessing aldehyde dehydrogenase 1 as a marker for normal and malignant human epidermal stem cells

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There is increasing evidence suggesting that malignant tumours originate from and contain a cellular subpopulation retaining key characteristics of tissue-specific stem cells. These properties include self-renewal, which drives tumour growth, and the ability to differentiate to generate tumour heterogeneity. This 'cancer stem cell hypothesis' has important clinical implications, suggesting that effective cancer therapies must target the rare stem cell population fuelling tumour growth. We are investigating the use of the detoxifying enzyme aldehyde dehydrogenase 1 (ALDH1) as a potential marker for normal and malignant human epidermal stem cells. Previous studies have shown that high ALDH1 activity identifies normal and malignant human breast and colonic stem cells and that expression correlates with poor prognosis in mammary carcinoma. We have used fluorescence-activated cell sorting to demonstrate the presence of ALDH1 activity in a subset of human epidermal keratinocytes, particularly within the stem cell enriched α_6 and β_1 integrin-high subpopulations. Immunoperoxidase labelling reveals localization of ALDH1 expression in specific keratinocytes in normal epidermis, squamous cell carcinomas and basal cell carcinomas. Finally, we have preliminary evidence that normal and neoplastic keratinocytes with ALDH1 activity show increased proliferation *in vitro*. In future studies, we will investigate whether ALDH1 is a *bona fide* marker of normal and malignant cutaneous stem cells. In addition, we will determine whether ALDH1 expression may serve as a prognostic indicator in cutaneous carcinomas and assess the functional consequences of ALDH1 depletion in normal and neoplastic keratinocytes. We anticipate that these experiments will contribute towards identifying novel therapeutic targets for treatment of cutaneous carcinomas.

P61
Do laypersons have intrinsic pattern recognition abilities that could be harnessed to allow the accurate and early diagnosis of skin cancers?

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Humans are able to classify visual objects almost effortlessly in the natural world without using explicit definitions of likeness or rule-based approaches (such as the ABCD), if provided with sufficient and appropriate training examples and feedback. We are interested in whether such cognitive abilities can be harnessed to improve lay and professional expertise. One fundamental question is whether, and to what extent, there is a degree of commonality between novices' classification of skin lesions. If there is little commonality our strategy is made more difficult; on the other hand, if different persons respond similarly to cognate clues then design of semiautomated systems is made easier. Can novices reliably identify similarities between different skin lesions and on that basis classify them? Twenty nonmedical novices were recruited. Twelve skin lesion images were selected based on a dermatologist's opinion of similarity. The 12 lesions consisted of three images from each of four diagnostic categories: haemangiomas, seborrhoeic keratoses, melanocytic naevi and basal cell carcinomas. Subjects compared each of the 12 images with the other 11. For each of these 66 pair-wise comparisons the subjects were asked to attribute a similarity score on a 7-point Likert scale (1 = very dissimilar, 7 = very similar). No definition of 'similar' was provided. The order of the comparisons was randomized. All comparisons were repeated. Pairs of images from the same diagnostic class had a median score of 7 and of different classes a median score of 1 ($P < 0{\cdot}0001$, Wilcoxon test). Intrasubject results were consistent between attempts, with a Spearman rank correlation of rho = 0·84 ($P < 0{\cdot}0001$). Intersubject results for each of the 66 comparisons were also consistent; Kendall's W was highly significant ($P < 0{\cdot}0001$). To obtain a graphical interpretation of the novices' similarity scores a two-dimensional nonparametric multidimensional scaling (MDS) model was derived. The model had an excellent fit with Kruskal's Stress of 7.46%. The MDS model demonstrated clustering of the 12 skin lesions similar to the dermatologist's original groupings and to the lesions' pathological diagnoses. Our results show that even novices have the ability to group skin lesions as similar, implying some commonality between persons. We would suggest that with an appropriate software environment this might prove medically useful.