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FOCUSING ON GENOMIC AND PHENOMIC ABERRATIONS IN NON-MELANOTIC SKIN CANCERS

N.A. Lintell*, D.J. Maguire*, L.R. Griffiths#, and M. McCabe* [1]

1. INTRODUCTION

Solar Keratosis is a precancerous lesion that is directly linked to long-term exposure to ultraviolet light (both ultraviolet B and A have been linked to DNA damage) [1]. These lesions have been categorised as Keratinocytic Intraepithelial Neoplasias or KIN. Solar Keratosis (SK) has three dermatologic stages, (I, II, and III), which the lesion progresses through before it is classified as a Squamous Cell Carcinoma (SCC) in situ [2]. The genes associated with SCC development that were analysed in this study are the MMP12, EMS1 and RASA1 genes[3,4]. Those associated with the progression of Basal Cell Carcinoma (BCC) are the PTCH and SMOH genes [3].

Mitochondria are the only extra-nuclear cellular organelle to contain their own DNA, and some of the genes it possesses encode several subunits of the ETC (13 out of the known 91)[5,6,7]. Mitochondria are also responsible for the initiation and regulation of apoptosis, which is a cellular process that removes diseased and damaged cells [5,6,7].

It is the malfunction of this pathway that has been fundamentally linked to the initiation of carcinogenesis [8]. The five genes that are involved in the ETC that were investigated are all encoded by the nucleus. They include NDUFA8, NDUFA5, and NDUFV1 from complex I, SDHD from complex II, and COXVIIc from complex IV[9].

This study analysed samples from a solar keratosis cohort via Real-Time PCR (polymerase chain reaction). This technique can measure the amount of target DNA (i.e. the gene that is being analysed) produced in real time as the reaction progresses[10]. It does this by detecting a particular compound that is bound to the DNA template (either a non-specific compound like SYBR Green I or a specific probe, such as a dual-labelled one), which fluoresces at a certain wavelength [11]. Each reaction cycle produces more DNA and the instrument gauges the total amount produced at the end of the PCR run [10]. Non-

[1] *School of Biomolecular and Biomedical Science and # School of Health Science, Griffith University Queensland 4111, Australia

specific compounds like SYBR Green I can also be used to calculate changes in DNA length by participating in melt-curve measurements, which utilises the fact that smaller DNA amplicons melt at a lower temperature than larger ones (if the G/C content is similar) [11].

2. METHODOLOGY

The first component of this study was to identify candidate genes. This process was aided by the submission of a PhD in 2002 by Kevin Ashton [12] who had analysed skin samples taken from BCC, SCC, and SK patients. He investigated these samples utilising the Comparative Genomic Hybridisation (CGH) technique, to analyse large areas on chromosomes for gains and losses. This study identified several areas of interest for the project to pursue, with the majority of these chromosomal areas associated with the location of NMSC and ETC genes. Indicated in Table 1 are the primer sequences devised for analysing specific exons of the genes in question. The study chose to analyse these gene sets via Real-Time PCR and SYBR Green I. The primers designed for each of the specific gene exons are depicted in Table 1, along with their associated PCR conditions.

Table 1. Primer sequences, MgCl2 concentrations, and melt temperatures

SDHD	Forward	AAGTAGCTTACCTATGGTCA	2.5uM, 56ºC
SDHD	Reverse	AAGCAGCAGCGATGGAGA	2.5uM, 56ºC
MMP12	Forward	CCATAGGTCATCTATTCTAG	2.5uM, 56ºC
MMP12	Reverse	ACGTTGGAGTAGGAAGTCA	2.5uM, 56ºC
NDUFA5	Forward	GGTAATATTTTAACCTATGG	3.0uM, 52ºC
NDUFA5	Reverse	TTGGTTAAATGTTACACAAG	3.0uM, 52ºC
SMOH	Forward	CCTAAGGTCACAGAATGGCC	3.0uM, 62ºC
SMOH	Reverse	CTGTACCTTCAGGTCTGGGT	3.0uM, 62ºC
NDUFA8	Forward	AGCAAGGCTATGTATTTGAG	3.0uM, 56ºC
NDUFA8	Reverse	TCTGTATTTACAGAGACCCT	3.0uM, 56ºC
PTCH	Forward	TTCATGGTCTCGTCTCCTAA	3.0uM, 58ºC
PTCH	Reverse	AAGTGAACGATGAATGGACA	3.0uM, 58ºC
NDUFV1	Forward	AGATCATCAGGCCCTCTCTT	2.5uM, 62ºC
NDUFV1	Reverse	CGCAGAAGGGTGGTGAATAC	2.5uM, 62ºC
EMS1	Forward	ATTGCTGCCCTGTCTCTCCA	2.5uM, 62ºC
EMS1	Reverse	AACGCGTGATTACAGACCGT	2.5uM, 62ºC
COXVIIc	Forward	CATCTGTCCTCATTCTCTGC	2.0uM, 60ºC
COXVIIc	Reverse	CCCTTACACACTAACCTTCC	2.0uM, 60ºC
RASA1	Forward	TTGTGAATCTGGTTTTAGGT	2.0uM, 54ºC
RASA1	Reverse	GTTTCTGTATCAACTTACAG	2.0uM, 54ºC

It was found that a standard stock of 1x BioTaq reaction buffer, 0.8 uM dNTPs, 0.5 uM primer and 1.5 units of BioTaq polymerase was needed for each of the primers as the majority of them had been specifically selected to have similar compositions and melt temperatures.

An initial denaturation step of 95ºC for three minutes was implemented for all the primer sets. The cycling component was comprised of a 95ºC step for 25 seconds,