TUMOUR-SUPPRESSIVE ACTIVITY OF
THE GROWTH ARREST-SPECIFIC
GENE, GAS1

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degree of Doctor of Philosophy

by

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SUMMARY

The growth arrest-specific gene, GAS1, codes for a membrane-associated protein which accumulates at the cell surface following growth arrest mediated by either serum starvation or density-dependent inhibition. Expression of both GAS1 mRNA and protein is rapidly down-regulated when cells are activated to enter S phase. Overexpression of the mouse gas-1 gene in normal and oncogene-transformed NIH3T3 cells blocks proliferation of actively-growing cells and inhibits entry by quiescent cells into S phase when activated by serum. The growth suppressing activity of the mouse gas-1 gene is mediated by a p53-dependent signalling pathway, but the transcriptional activation function of p53 is not required for gas-1-induced growth arrest. Since the expression of the GAS1 gene is tightly confined to the quiescent stage of the cell cycle and GAS1 has growth-inhibitory activity in-vitro, its expression either reflects requirements to sustain growth arrest, or may be involved in the control of differentiation.

Gene products with growth-inhibitory properties frequently act as tumour suppressor genes and the studies described in this thesis seek to establish a possible role for GAS1 as a candidate tumour suppressor gene whose function is to inhibit uncontrolled proliferation and to prevent the development of the malignant phenotype.

The first part of this thesis reports on the chromosomal localisation by in-situ hybridisation of the mouse and human GAS1 genes. The mapping of GAS1 to mouse Chromosome 13 at bands B3-C2 and, more particularly, to human chromosome 9 at bands q21.3-q22, places the human gene in a region of the genome which is highly significant as a site of a number of potential tumour suppressor genes. Genetic
alterations in this region of chromosome 9q have previously been implicated as early events in the progression of myeloid malignancy and bladder carcinoma.

The human GAS1 gene, when overexpressed in NIH3T3 cells, is able to block proliferation of actively growing cells. Cells overexpressing exogenous GAS1 display remarkable changes in phenotype that resemble growth arrested cells or cells in crisis. These results clearly demonstrate that the human GAS1 gene product is directly involved in mediating growth suppression in a similar manner to that previously observed with the mouse homologue.

To investigate whether GAS1 is able to suppress the growth of human tumours, GAS1-negative tumour cells were transfected with GAS1-expressing plasmids and the growth characteristics of stable transfectants were analysed. The A549 lung adenocarcinoma cell line contains wild type p53 and normal levels of the MDM2 oncogene product. When a constitutively-expressing GAS1 plasmid was transfected into A549 lung adenocarcinoma cells, no stable clones expressing GAS1 were isolated. When A549 cells were transfected with a dexamethasone-inducible GAS1 plasmid, expression of GAS1 inhibited growth in vitro and produced fewer slow-growing tumours in nude mice. GAS1 also inhibited proliferation of an HT1080 fibrosarcoma subline with wild-type (wt) p53 and normal levels of MDM2. In contrast, when the HT1080 subline, HTD114, was transfected with the constitutive GAS1 plasmid, there was no reduction in colony number. GAS1-transfectant clones had unaltered growth in vitro, and showed no difference in their ability to form tumours in nude mice. Although HTD114 cells contain wt p53, levels of MDM2 were elevated by 10-15 fold. The product of the MDM2 gene physically associates with wild type p53 protein and inhibits its transactivation function. Overexpression of MDM2 therefore provides an alternate mechanism by which p53 function is inhibited. The HT1080/STGc5 subline with mutant
p53 and normal levels of MDM2 was also refractory to GAS1. These results indicate that GAS1 suppresses the growth and tumorigenicity of tumour cells in a p53 dependent manner and that p53 mutation and/or overexpression of MDM2 abrogates the GAS1-mediated growth-suppressing pathway.

To further define the role of MDM2 in the inhibition of GAS1-mediated growth suppression, the endogenous levels of MDM2 in the non-responsive HTD114 cells were downregulated using an antisense strategy. HTD114 cells were co-transfected with MDM2-antisense and GAS1 plasmid constructs. If overexpression of MDM2 in HTD114 cells inhibits the suppression of growth mediated by GAS1, then downregulation of the endogenous levels of MDM2 in these cells using the antisense construct, should increase the sensitivity of the co-transfectants to exogenous GAS1. Although there was a reduction in the levels of endogenous MDM2 mRNA, these cells remained refractory to overexpression of GAS1. However, these cells now expressed a previously unrecognised and novel MDM2 splice variant, which, by sequence analysis, was shown to contain most of the p53 binding domain and was devoid of the putative DNA binding motifs present in the carboxy end of the MDM2 gene. Based on these observations, the following model is proposed. The net effects of antisense MDM2 changes the balance in favour of free and functional p53 protein within the cell and that these cells now overcome the GAS1-mediated growth suppression by expressing the alternate splice variants of MDM2, thus maintaining the cells in a transformed state.

Taken together, the results presented in this thesis have established the growth-suppressive activity of the human GAS1 gene and provided the first direct evidence that GAS1 can inhibit the growth of tumours. The antiproliferative effects of GAS1 are mediated by a p53 dependent pathway. Functional inactivation of p53 by either p53 mutation and/or overexpression of the MDM2 oncogene product abrogates GAS1-
mediated growth-suppression. This study has therefore identified another downstream element, MDM2, that is involved in the cascad of events that lead to growth arrest mediated by GAS1. Further characterisation of the previously unrecognised alternate splice variant of MDM2 identified in HTD114 cells presents a challenge of defining its role and oncogenic potential.