Gene induction by desiccation stress in human cell cultures

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Abstract One strategy for investigating desiccation tolerance is to use mammalian cells, which are sensitive to desiccation, as a model for testing putative adaptive mechanisms. However, how mammalian cells themselves respond to desiccation is poorly characterised. Although MAPK signal transduction pathways are activated by desiccation of human cells, hypertonicity-responsive genes *AR*, *BGT1* and *SMIT* are not significantly induced, although they are proposed to be regulated by physiological changes which should occur during drying. To determine whether a response to desiccation occurs at the transcriptional level in human cells, we performed genome-wide microarray analysis. Twenty upregulated genes, including early stress response and transcription factor genes, were identified, most of which, e.g., *EGR1*, *EGR3*, *SNAI1*, *RASD1* and *GADD45B*, were also induced by hypertonicity, indicating common regulatory mechanisms. Our data suggest that human cells can initiate a complex desiccation stress response distinct from, but overlapping with, that to hypertonic stress.

Keywords: Desiccation; Desiccation response; Hypertonicity; Anhydrobiosis; Mitogen-activated protein kinase

1. Introduction

Water is essential to life, but certain organisms (e.g., rotifers, nematodes, and tardigrades) can survive almost complete desiccation by entering anhydrobiosis, a unique dry state of reversible metabolic arrest. As a survival strategy in nature, anhydrobiosis involves morphological and physiological adaptations to desiccation, but the molecular mechanisms governing the adaptative responses are far from fully understood [1]. Definition of desiccation-induced genes in anhydrobiotic organisms should identify key adaptations and thus provide a gene set for potential use in anhydrobiotic engineering, which aims to confer desiccation tolerance on otherwise sensitive cells or organisms [2]. A number of advantages have made mammalian cell cultures the model of choice for anhydrobiotic engineering, including ease of growth and modification, well-characterised cell physiology, genetics and biochemistry, and potential biomedical applications of desiccation-tolerant cell types (e.g., for biosensors or tissue engineering; [2,3]). Surprisingly, however, little information is available on the response of mammalian cells to desiccation, even though such information is fundamental to research in this field.

We have recently shown that a human cell line derived from embryonic kidney, which is not desiccation tolerant, is capable of responding to desiccation stress via rapid activation of JNK and p38 mitogen-activated protein kinases (MAPKs), within the same time-scale as the response to hypertonic stress, which also results in MAPK activation [4]. One of the most important functions of MAPK signalling networks is to modulate gene expression in response to pathophysiological stimuli and environmental stresses [5]. However, although similar MAPK activation profiles result from desiccation and hypertonic stress, gene induction patterns differ: three genes which are upregulated by hypertonicity – *AR* (aldose reductase), *BGT1* (betaine-γ-amino-*n*-butyric acid transporter) and *SMIT* (sodium-dependent *myo*-inositol transporter), all of which govern organic osmolyte accumulation – are not induced by desiccation [4]. The difference in the transcriptional response of these genes to the two closely related dehydration stresses is puzzling because, in osmotically stressed cells, their expression is reported to be regulated by changes in intracellular ionic strength and cell volume due to osmotic efflux of water [6]. Changes in ionic strength and cell volume are also expected to occur in drying cells suffering evaporative water loss, but since *AR*, *BGT1* and *SMIT* are not upregulated by desiccation, it is likely that other factors are also involved [4,7].

To improve our understanding of the response of mammalian cells to desiccation stress, and specifically to determine whether mammalian cells are capable of responding at the level of gene expression, we performed cDNA microarray analysis and quantitative real-time PCR to identify desiccation-responsive genes. We found that most of the desiccation-sensitive genes were also induced by hypertonicity imposed by a range of osmotica (NaCl, disaccharides and sugar alcohols), suggesting significant overlap in the transcriptional response to osmotic and evaporative water loss, at least in the early stage of the stress response.

2. Methods

2.1. Cell cultures and stress treatments

Human embryonic kidney cell line, T-REx 293, was maintained as described [4]. Cells were grown to near confluence in air-vent culture dishes prior to stress treatment, which was performed on the same number of cells in an identical fashion in each experiment. For desiccation, medium was first carefully removed by two sequential pipetting operations, with a brief draining period in between when the dish is tilted on its side. Then cells were placed in a saturated $CuSO_4$ humidity chamber (98% relative humidity; RH) and dried at 37 °C for indicated times. Thus, although cell water content was not determined directly, we expect that drying occurred in a similar fashion from experiment

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to experiment. For hypertonic shock and starvation experiments, near-confluent cell cultures were further incubated at 37 °C for indicated times after the medium was replaced with (a) fresh medium (normal medium control), (b) medium containing 100 mM NaCl or 200 mM trehalose, sucrose, mannitol or sorbitol (hypertonic stress), and (c) medium without serum or glucose (starvation).

2.2. Microarray analysis

Near confluent T-REx 293 cells in tissue culture petri dishes (90 mm) were partially dried as above at 37 °C for 8 h. Total RNA was prepared from control and dried cells using the RNAeasy kit (Qiagen, Hilden, Germany). After reverse transcription and labelling, the resulting cDNA mixture was hybridised with Human Known Gene SGC Oligo Set Arrays (~19k oligos; produced by the Human Genome Mapping Project Resource Centre, Hinxton, UK) using the facilities at the MRC Mammalian Genetics Unit, Harwell, UK. Microarray data were analysed using GeneSpring software (Silicon Genetics, Redwood City, CA); details given in [8].

2.3. Quantitative real-time PCR

Real-time PCR analysis was performed essentially as described [4]. Briefly, T-REx 293 cells were grown in 0.5 ml medium in multidishes (1.9 cm^2/well), and cDNA was prepared using the Cells-to-cDNA II kit (Ambion, Huntingdon, UK) and diluted 10–20 times with water. Relative quantification of cDNA was performed using a Rotor-Gene RG3000 (Corbett Research, Sydney, Australia) and QuantiTect SYBR Green PCR Kit (Qiagen). The 18S rRNA gene transcript was used as a reference gene in addition to *ACTB* (β-actin) and *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) as previously. Primers of target gene transcripts are listed in Supplementary Material.

3. Results

3.1. Genes induced by desiccation stress

To identify genes responsive to desiccation stress, we employed cDNA microarray analysis using RNA samples from control and dried T-REx 293 cells; ~50% of the cells survived the drying conditions (98% RH at 37 °C for 8 h; [4]). The microarray experiment was performed in triplicate (with a biological and a technical replicate), and transcripts showing more than 2-fold induction or suppression in all three replicates were considered as candidate genes responsive to desiccation (Table 1). Notably, several of the genes identified were early response genes and/or zinc finger transcription factors, e.g., *IER3*, *EGR1*, *EGR3*, *SNAI1* and *CITED2*. Other candidate genes identified, with functions of potential relevance to a desiccation response, are: *SGK* [9], regulation of cellular hydration and cell volume; *CTGF* [10] and *THBS1* [11], cell–extracellular matrix interactions; and *SNK* [12] and two downregulated genes, *CDCA4* and *CDK5RAP3*, cell cycle regulation.

3.2. Time course of gene induction by desiccation stress

Most of the annotated desiccation-responsive genes identified by microarray analysis were further examined by quantitative real-time PCR analysis after T-REx 293 cells were dried for different times. The threshold cycle number (Ct) for control and desiccation samples was very close for *ACTB* and *GAPDH* reference transcripts, respectively, when cells were dried for up to 12 h, but Ct increased when cells were dried for longer periods, probably due to decreased cell viability. The Ct of another reference transcript, 18S rRNA, was similar between control samples and samples dried for up to 6–8 h but again increased after longer drying times. *ACTB* and *GAPDH* were used routinely as the reference genes in our studies for cells dried for up to 12 h (~50% viability). Where tested, i.e., for 16 out of 20 upregulated genes and one of the six downregulated genes, real-time PCR results agree with microarray data and confirm that the genes identified are regulated by drying.

Table 1
Desiccation-responsive genes identified by cDNA microarray analysis

Gene	Description	Accession No.
Upregulated genes (>2-fold in all replicates)		
IER3 (*IEX-1*)	Immediate early response 3; immediate early gene X-1	NM_003897
EGR1	Early growth response 1	NM_001964
EGR3	Early growth response 3	NM_004430
SNAI1	Snail homolog 1 (*Drosophila*)	NM_005985
FOXG1B	Forkhead box G1B	NM_005249
RASD1	RAS, dexamethasone-induced 1	NM_016084
THBS1 (*TSP1*)	Thrombospondin 1	NM_003246
CTGF	Connective tissue growth factor	NM_001901
CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	NM_006079
ADM	Adrenomedullin (ADM precursor)	NM_001124
CRSP7	Cofactor required for Sp1 transcriptional activation, subunit 7	NM_004831
CXCR4	Chemokine (C–X–C motif) receptor 4	NM_003467
GADD45G	Growth arrest and DNA-damage-inducible	NM_006705
SGK	Serum/glucocorticoid regulated kinase	NM_005627
SNK (*PLK2*)	Serum-inducible kinase; polo-like kinase 2 (*Drosophila*)	NM_006622
ANKRD10	Ankyrin repeat domain protein 10	NM_017664
NPD014	NPD014 protein, transcript variant 2, mRNA	NM_020317
–	Hypothetical protein	AL049299
–	T cell mRNA, 126 bp	Z36505
–	mRNA for KIAA1321 protein, partial cds	AB037742
Downregulated genes (>2-fold in all replicates)		
CDCA4	Cell division cycle associated 4	NM_017955
CDK5RAP3	CDK5 regulatory subunit associated protein 3	AF110322
–	Human XIST, coding sequence "d" mRNA	X56196
–	Human XIST, coding sequence "c" mRNA	X56197
–	Full length insert cDNA clone YP24E09	AF085880
–	mRNA from HIV-associated non-Hodgkin's lymphoma	Y17179
–	cDNA FLJ10071 fis, clone HEMBA1001702	AK000933