Role of p90<sup>RSK</sup> in regulating the Crabtree effect: implications for cancer

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Abstract

High glucose inhibits mitochondrial respiration, known as the ‘Crabtree effect’, in cancer cells and possibly other cell types. The upstream pathways regulating this phenomenon are poorly understood. In diabetes, where glucose levels are elevated, the p90<sup>RSK</sup> (p90 ribosomal S6 kinase) has received much attention as a potential upstream mediator of the effects of high glucose. Evidence is also emerging that p90<sup>RSK</sup> may play a role in cancer cell signalling, although the role of p90<sup>RSK</sup> in regulating cancer cell metabolism is unclear. In the present paper, we provide an overview of the Crabtree effect and its relationship to mitochondrial metabolism. Furthermore, preliminary data are presented suggesting a role for p90<sup>RSK</sup> and its upstream components, the ERK (extracellular-signal-regulated kinase) family of MAPKs (mitogen-activated protein kinases), in the Crabtree effect.

Introduction

The exposure of cells to high concentrations of glucose results in a number of physiological and pathological responses. In the field of diabetes research, the effects of glucose on mitochondrial ROS (reactive oxygen species) generation have been well studied, and are believed to underlie much of the secondary pathology of diabetes, particularly vascular complications [1]. In the field of cancer research, the effects of glucose on cancer cell metabolism have also been studied extensively, but the majority of attention has been given to the Warburg effect; the phenomenon in which cancer cells appear to prefer anaerobic glycolysis as a source of ATP even in the presence of adequate oxygen for mitochondrial OxPhos (oxidative phosphorylation) to occur [2,3].

In contrast, the Crabtree effect, postulated in 1929, has languished in the shadow of the Warburg effect in terms of research attention [4]. The Crabtree effect is simply defined as the observation that high concentrations of glucose result in a decrease in mitochondrial respiration in cancer cells [4]. The role of inhibited mitochondrial function in the Warburg effect is still unclear [5], and as such it is possible that the Crabtree effect may be an underlying cause of the Warburg effect. The present paper describes evidence favouring a role for p90<sup>RSK</sup> in the Crabtree effect, and discusses this in the context of cancer signalling.

ERK5/MEK5/p90<sup>RSK</sup> pathway in cancer

MAPKs (mitogen-activated protein kinases) are essential components in many cell signalling events including proliferation, differentiation and cell death. The biological actions of ERK (extracellular-signal-regulated kinase) 5 [also known as BMK1 (big MAPK1)] are well described [9,10] in several cell proliferative and developmental pathways, as well as cancer. MEK (MAPK/ERK kinase) 5 [10,11] has been closely linked to ERK5, and nearly all cellular processes in which MEK5 has been implicated are attributed to regulation by ERK5.

Within the field of cancer research, the MEK5/ERK5 pathway has been implicated in cell growth control [12], and a role for ERK5 has been postulated in EGF (epidermal growth factor)-induced proliferation and cell-cycle progression [13]. Furthermore, studies in ERK5-knockout mice show a crucial role for ERK5 in angiogenesis and vascular cell growth [14], especially as a modulator of other antigenic signals.

In patients suffering from multiple myeloma, ERK5 is expressed in B-cells [15] and it is constitutively activated in breast cancer cells overexpressing the ErbB2 receptor [16]. ERK5 activation is required for the proliferation of myeloma and breast cancer cell lines in response to IL-6 (interleukin-6) and neuregulins respectively. Cyclin D1, whose deregulation is implicated in a wide variety of cancers, was identified as a target of the MEK5/ERK5 pathway [17]. The authors showed that the kinase activity of ERK5 is required for induction of cyclin D1 in response to
serum in some breast cancer cell lines [17]. In prostate and breast cancers, MEK5 overexpression is associated with poor survival and resistance to chemotherapy respectively [18,19]. Furthermore, prostate cancer cell growth can be suppressed by inhibition of MEK5 signalling, through the enhanced degradation of the protein [20]. ERK5 and p90RSK have some overlapping functions in cancer: p90RSK similar to ERK5 is thought to stimulate cell-cycle progression [21]. ERK5 also responds to cAMP [22] and plays a role in signalling to the pro-apoptotic protein BAD (Bcl-2-associated death promoter) [23], which is a direct substrate of p90RSK [24]. Overall, there are several lines of evidence linking p90RSK to cancer and to glucose sensing, but surprisingly the role of this serine/threonine kinase in mediating the Warburg or Crabtree effects has not been investigated.

**Experimental**

The metabolic function of the H9c2 cardiomyocyte cell line was measured using an XF24 extracellular flux analyzer (Seahorse Bioscience) [25,26]. Cells were incubated in a glucose-free Krebs–Ringer solution (Hepes, pH 7.3) for 30 min, with or without 20 mM glucose, with the addition of kinase inhibitors and other pharmacologic agents, as follows: (i) BIX02188 (MEK5 inhibitor, 20 μM); (ii) UO126 (MEK1/2 inhibitor, 10 μM); (iii) FMK (fluoromethylketone-pyrrolopyrimidine, p90RSK inhibitor [21], 10 μM); and (iv) splitomicin (SIRT1 inhibitor, 10 μM).

Figure 1 shows that H9c2 cells exhibited a robust Crabtree effect, with 30 min exposure to 20 mM glucose resulting in a 20% decrease of cellular oxygen consumption rate. These data are in broad agreement with those of Sweet et al. [6], who reported a robust Crabtree effect in endothelial cells.

Thus the Crabtree effect is not unique to cancer cells, and may apply to many types of dividing cells. Notably, this effect was blocked by pre-incubation with the p90RSK inhibitor FMK (Figure 1A) or the MEK5 inhibitor BIX (Figure 1B). Other pharmacophores tested included the MEK1/2 (ERK1/2) inhibitor UO126, or the SIRT1 inhibitor splitomicin, but neither had a significant effect on the inhibition of respiration by glucose (results not shown).

**Discussion**

The role of MAPKs in the response to glucose in general has been well studied, and it was shown that some mitochondrial responses to high glucose could be blocked by high concentrations of the non-specific MEK1/2 (ERK1/2) inhibitor PD98059 [27], although this effect may have been due to the off-target effects of PD98059 [28].

In previous reports, attention within the MAPK field has focused on another member of the ERK family, ERK5 (BMK), which is an upstream mediator of p90RSK activity [29,30]. This has been facilitated by the commercial availability of BIX02188, an inhibitor of MEK5, which is the kinase immediately upstream of ERK5. Our data in Figure 1(B) suggest that indeed MEK5, through ERK5, is an upstream kinase involved in the activation of p90RSK in response to high glucose. This signalling pathway is shown in Figure 2, suggesting key roles for both MEK5 and p90RSK in the Crabtree effect.

To the best of our knowledge, this is the first identification of any signalling pathway involved in mediating the Crabtree
effect. Notably, p90Rsk has been linked to signalling by the tumour suppressor protein p53 [31]. In addition, p53 is emerging as an important regulator of mitochondrial function [32–34]. Thus p90Rsk may play additional roles in cancer signalling beyond the Crabtree effect, as reviewed above.

The relationship between the Crabtree effect and mitochondrial morphology is also worthy of further consideration. Specifically, a link has been established between mitochondrial fragmentation and ROS generation in response to high glucose [27,35]. Thus it is interesting to speculate that mitochondrial fragmentation may be an intermediate step between p90Rsk and the inhibition of respiration. Clearly, the elucidation of this complex signalling pathway, which drives the response of cancer and other dividing cell types to high glucose, may lead to the development of novel therapeutic approaches, based on the inhibition of p90Rsk and its upstream signals.

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**References**


