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Expression of stress response protein glucose regulated protein-78 mediated by c-Myb

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Abstract

Glucose regulated protein-78, GRP78 has been implicated in the protection of tumor cells from cytotoxic damage and apoptosis. When protein profiles of colon cell lines were investigated we found remarkably high GRP78 expression in two cell lines. These cell lines express elevated levels of the transcription factor c-Myb due to genomic amplification of the *c-myb* locus and we hypothesized that c-Myb regulates GRP78 expression in colon cancer cells. The promoters of human and murine *GRP78* and the related family member *GRP94* were examined and potential c-Myb binding sites were identified and characterized. DNA binding studies with recombinant c-Myb and nuclear extracts together with ChIP assays on colon cell lines validated these sites. Endogenous *GRP78* expression was further induced in these colon cells in response to Thapsigargin treatment, a potent inducer of the unfolded protein response. Transactivation studies with the human *GRP78* promoter in colon cell lines showed reporter activity was dependent upon the presence of a conserved c-Myb binding site independent of sequences associated with the unfolded protein response. Finally, over-expression of c-Myb induced the endogenous *GRP78* gene. These data suggest that amplification of *c-myb* in tumor cells may lead to robust *GRP78* gene induction, which may in turn assist cells in survival under conditions of oxygen deprivation and nutrient stress.

Keywords: c-myb; GRP78; BIP; Unfolded protein response

1. Introduction

Expression of the heat shock protein family members including Hsp70, GRP78/Bip and GRP94 is essential for normal cell physiology (Gething, 1999).

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Modest expression of GRP78 and GRP94 is required for protein folding in most cells and appears to play a particularly important role in B-cells where immunoglobulin secretion is dependent upon GRP78/Bip function (Munro & Pelham, 1986).

c-Myb is a highly conserved transcription factor required for normal colon development and hematopoiesis (Mucenski et al., 1991; Zorbas et al., 1999). We have investigated the progression of colorectal cancers (CRC) and have consistently found an increase in c-Myb expression from normal tissue to pre-malignant polyps, primary (Ramsay et al., 1992) and finally metastatic colon tumors (Biroccio et al., 2001). Colon tumors and derived cell lines frequently over-express c-Myb (Alitalo et al., 1984; Ramsay et al., 1992; Torelli et al., 1987; Trainer et al., 1988). In our examination of highly metastatic cell lines we noted an elevated level of GRP78 protein. Co-incident with this high GRP78 expression was the high expression of the transcription factor c-Myb due to *c-myb* amplification (Alitalo et al., 1984). Elevated expression of GRP family members is a common feature of transformed cells (Bini et al., 1997; Gazit, Lu, & Lee, 1999; Menoret, Meflah, & Le Pendu, 1994; Patierno, Tuscano, Kim, Landolph, & Lee, 1987). Recent observations have connected the regulation and expression of GRP78 with resistance to apoptosis (Bernstein et al., 1999; Jamora, Dennert, & Lee, 1996; McCormick, McColl, & Distelhorst, 1997; Reddy, Lu, & Lee, 1999; Reddy et al., 2003; Sacchi & Schiaffonati, 1996; Suzuki, Tomida, & Tsuruo, 1998) and over-expression of GRP78 appears to potentiate tumor progression (Jamora et al., 1996). Protection from stress induced by oxygen and glucose deprivation, cytotoxic cell killing and more generally apoptosis are likely to give tumor cells a selective advantage particularly at a site anatomically distant from the primary tumor (Koong, Chen, Lee, Brown, & Giaccia, 1994).

The propensity to induce angiogenesis represents part of the response of tumors to oxidative and nutrient stress (Brown & Giaccia, 1998). However, in order to survive until new vessels provide a nutrient supply and oxygenation, other immediate responses are required. These might be expected to include induction of genes like the *GRP78/94* family associated with protection from apoptosis. Therefore understanding the regulation of this class of genes may offer valuable insights into potential therapeutic targets like the newly discovered compound *Versipelostatin*, for the control of malignant progression and metastasis (Park et al., 2004). The study reported here demonstrates that c-Myb can bind to sequences within, and transactivate, the human *GRP78* promoter. In addition c-Myb directly activates transcription of the *GRP78* promoter through conserved sequences independent of the unfolded protein response (UPR). Together the data support the concept that induction of target genes like *GRP78* may serve as the basis for selection of colon tumors with amplified *c-myb* giving tumor cells a selective survival advantage.

2. Experimental procedures

2.1. Cell lines

Colon carcinoma cell lines Colo201, Colo205, LIM2405, LIM2412 and LIM1215 (Thompson, Flegg, Westin, & Ramsay, 1997; Thompson et al., 1998) and HEK293 cells (Graham, Smiley, Russell, & Nairn, 1977) have been described previously. All cells were cultured in RPMI1640 supplemented with 10% FCS. Thapsigargin (Sigma) was used at 10^{-6} M and added 5 h prior to cell harvesting.

2.2. Preparation of protein

Protein was extracted from cell pellets (approximately 10^7 cells) by lysing samples on ice in 0.5% Nonidet P-40 (Sigma), 2 mM $MgCl_2$, 5 mM KC1, 3 mM $CaCl_2$, 10 mM Tris–HCl, pH 8, 10% glycerol, 1 mM Trazylol (Bayer) 1 mM phenylmethylsulfonyl-flouride (Sigma), 1% sodium dodecylsulphate (SDS) and 500 mM NaCl. After 5 min insoluble material was pelleted by centrifugation at 26,000 rpm in a Sigma 3K30 Ultrafuge (Sigma, Germany) at 4 °C for 15 min. The supernatant was then removed and the amount of total soluble protein determined using a BioRad protein assay. Two-dimensional SDS-PAGE has been described previously (Anderson, van Kersen, Kraft, & Hahn, 1989).