Cell culture provides the opportunity to study and manipulate cells in-vitro which would other wise be impossible to undertake in-vivo. In establishing and maintaining cell cultures the primary criteria relates to growth, morphology and response to experimental manipulation. However, it is well established that during culture apparently normal cells may develop varying responses to agents. Podansky et al identified altered glycosylation of insulin and insulin-like growth factor receptors in a Chinese hamster ovary cell-line, which caused binding specificity to change for insulin (1). Similar early research identified in-vitro induced modification of cells such as fetal bovine serum (FBS) concentration modifying cell glucose content and choice of buffer influencing glycosylation (2). Changes in glycosylation patterns have also been demonstrated between serum free media and media with FBS (3).

In the present research we wished to use a proteomics approach to identify whether proteins were affected by a simple modification of the FBS concentration in the culture media using 2-D gel electrophoresis.

Dulbecco’s Modified Eagle Medium (DMEM) with high glucose (4500mg.L\(^{-1}\)), L-glutamine and pyridoxine hydrochloride, without sodium pyruvate was obtained from Invitrogen, NZ. The antibiotics added to the culture media were, penicillin G, streptomycin sulphate and gentamicin sulphate (Sigma cell culture grade, Sigma, St Louis, USA). Fetal bovine serum was obtained from Invitrogen (NZ) and was all the same lot number for these experiments. The cells used were 3T3-L1 pre-adipocytes and were cultured to confluence initially in DMEM containing 10% FBS in an atmosphere of 5% carbon dioxide in air at constant humidity and 37°C. Media was changed every second day.

At confluence the cells were washed in protein free media and sub-passaged into one of three DMEM media containing 5%, 10% and 15% FBS respectively and again cultured to confluence. At confluence each of the three cultures were harvested using 0.067% trypsin solution in sterile Dulbecco’s A PBS (Invitrogen, NZ), washed in protein-free media, then resuspended in protein-free media at constant volume and cell counts performed. The cells were then adjusted to constant cell number and pelleted by centrifugation, washed three times in sterile Dulbecco’s A PBS then the final pellet was suspended in Dulbecco’s A PBS (50 to 100uL) containing 5% phenyl methyl sulphonyl fluoride (PMSF). This was then sonicated on ice for 10 minutes, then centrifuged and the supernatant either analysed immediately or stored at -20°C until analysis.

Prior to electrophoresis the total protein concentration of each cell homogenate was determined spectrophotometrically in triplicate using the BCA assay (4). Two-dimensional protein electrophoresis was undertaken using constant protein loading as previously described (5,6) using an ampholyte pH range 3.5 to 10.0. Five gels were run in duplicate for each FBS concentration. Staining was with 0.025% Coomassie Brilliant Blue R (Sigma Chemical Company, St Louis, USA) and once de-stained were dried onto cellophane for analysis by densitometry.

Overall there was no change in expression for the majority of proteins identified on the gels. However, each of the FBS concentrations did modify the expression of a small number of low molecular proteins. A summary of the overall changes in protein expression for the three FBS concentrations is shown in Table 1. Most notable was the absence of proteins in the pl 4.5 to 4.7 and molecular weight 42kDa to 47kDa ranges for 5% FBS and a decrease in expression in the 15% FBS. In addition, cells in the 15% FBS demonstrated synthesis of two proteins not identified in the other two FBS concentrations, (pl 4.7, M,80kDa and pl5.0, M,92kDa).

Using a proteomics approach we have identified specific changes in protein expression in cell culture, which were FBS concentration specific. This information indicates that the simple choice of FBS concentration will change the response of cell protein synthesis, which in turn has the potential to influence the outcome of the cell culture results. We did not investigate different batches of FBS, which can be notoriously fickle in promoting cell growth and proliferation. However, as the only variable in this work was the FBS, the results obtained are the consequence of FBS concentration, which may in turn be reflected in differing batches of FBS.

We used 10% FBS as the reference concentration and in comparison identified three low molecular weight proteins were missing or had reduced expression from both 5% and 15% FBS media respectively. At present we can only surmise that the low concentration of FBS may have protein or ‘factors’ at a concentration too low to promote the synthesis of the missing proteins and the converse for the 15% FBS may have an inhibitory effect. Using the pl and molecular weight of the three missing proteins the most likely candidate is actin (swissprot@www.expasay.org) a major cytoskeleton protein. In addition the likely candidate proteins identified in the 15%
FBS but not in the other two FBS concentrations matches stress proteins. We have not, however, taken the identification of these further at this stage.

In summary, it has been demonstrated that the concentration of FBS has the potential to modify protein synthesis of at least five proteins in-vitro. We have not attempted to dissect the causative agent for this effect but we have identified (data not shown) that cell surface glycosylation patterns are also influenced by FBS concentrations, particularly the synthesis of 2-0-linked α-L-fucosyl units. This may indicate that there is an overall effect on the cell metabolic machinery by FBS, which may in turn provide some clues as to the reason various batch of FBS are problematic in cell culture.

References


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