Culture conditions for cell lines are known to affect gene expression (1–3), while stem cells grown in different types of serum exhibit variable differentiation and proliferation characteristics (4,5). Yet often, minimal attention is paid to the selection of FBS for growing in vitro cultured cell lines. Researchers are usually concerned only that their cells are growing at optimal rates and without phenotypic changes. However, alteration to other more subtle cellular processes may explain why some laboratories obtain different results using the same cell lines grown under superficially similar conditions. Our laboratory observed that changing to a different FBS source influenced our experimental data obtained from gene reporter assays. We investigated this further by determining the selenium concentrations of each serum and analyzing various parameters, including gene promoter activity, cell proliferation, and antioxidant enzyme activity of cells grown in medium supplemented with different serum.

It is now well established that redox control systems play an important role in regulating numerous cellular functions, including gene expression and cell signaling (6–8). If these major redox systems are activated differently in cells grown in different media, then there are downstream consequences. For example, many transcription factors are redox-regulated, which in turn will regulate the activity of other gene promoters (9–14). Therefore, the use of cell lines in gene reporter assays, mRNA quantitation, Western blot analysis, and other functional assays may yield conflicting data depending on the choice of culture medium and serum.

The availability of more economical sera, including serum supreme (an iron-supplemented calf serum), as well as the sourcing of FBS from multiple countries, means that different chemical compositions are present in the sera used for routine cell culture. While all sera are tested prior to shipping, one analysis not routinely provided is the selenium concentration present in each batch of serum. Selenium is required for functionality of both the thioredoxin (Trx) and glutaredoxin redox control systems, since the key enzymes thioredoxin reductase (TrxR) and glutathione peroxidase (GPx) contain a selenocysteine residue in their active site (15–17). These systems have significant downstream effects on many cell-signaling pathways and on the activity of several transcription factors (9–14,18). Therefore, the selenium concentration in the cell growth medium may have significant consequences for gene transcription and protein activity.

Reporter assays are commonly used to measure gene promoter activity and to assess the response of a gene promoter to various stimuli. Problems can arise when other researchers repeat these experiments and obtain different levels of gene expression or different induction levels. Our laboratory has worked with the Trx gene promoter for many years (19,20), which is upregulated by many oxidative stress stimuli due to the cell’s requirement for Trx to maintain cellular redox homeostasis (21–23). Increased expression of Trx, as well as many other gene promoters, in response to oxidative stress, is mediated by nuclear factor (erythroid-derived 2)–like 2 transcription factor (Nrf-2) (21). We observed that luciferase reporter assays performed using the same cell line grown in media supplemented with different serum yielded significantly different results. Previously, when we stimulated the Trx gene promoter with a known oxidative stress–causing agent, tBHQ (19,22), we obtained an induction fold of >10-fold

**Figure 1. Luciferase activity in cells grown in medium supplemented with either SS or FBS.** Luciferase reporter assays were performed on lysates from MDA-MB-231 cells transfected with Trx promoter–luciferase constructs or the control pGL3-basic plasmid (A) or with the HRE-luciferase construct or control pGL3-promoter plasmid (B). Cells were grown in medium supplemented with either SS or FBS as indicated. Selenium concentrations (in μg/L) are shown in parentheses for each serum. Values shown are the average fold induction of luciferase activity from each reporter construct in response to treatment with 150 μM tBHQ for 20 h (A) or 0.1% oxygen (hypoxia) (B) for 16 h, calculated from luciferase expression in untreated cells. Three independent experiments were each conducted in triplicate, with luciferase activity measured in duplicate from each sample. Data are expressed as the mean ± s.d. Single asterisk indicates samples that show statistically significant differences from all other samples in the same panel (P < 0.05); double asterisk indicates samples that show a statistically significant difference from all other samples except for the Inv-US sample; triple asterisk indicates samples that show a statistically significant difference from all other samples except for the Lonza sample, as determined using an ANOVA followed by post hoc comparisons using Tukey’s procedure.
Figure 2. Enzyme activity in cells grown in medium supplemented with either SS or FBS. Enzyme activity assays were performed on cell extracts of MDA-MB-231 cells grown in media containing either Inv-AUS FBS (blue) or SS (red) without or with selenium supplementation (25–200 nM) as indicated. The assays performed measured the specific activity of TrxR (A), GR (B) and GPx (C). Data are expressed as specific activity (units/mg) and as the mean ± s d of three experiments analyzed in triplicate. A single asterisk represents a statistically significant difference between FBS and SS with no added selenium ($P < 0.01$), as determined using an independent t-test. A double asterisk represents a statistically significant difference between untreated cells and cells grown in selenium ($P < 0.01$), as determined using an ANOVA followed by post hoc comparisons using Tukey’s procedure.

in MDA-MB-231 breast cancer cells (24) grown with FBS sourced from Invitrogen. When the same cells were cultured in media supplemented with serum supreme (SS), the induction fold was <3-fold. We therefore decided to investigate this difference.

A range of FBS, sourced from various countries, was obtained from several companies. Since the Trx promoter was stimulated differently by an oxidative stress stimulus, we initially surveyed serum datasheets for differences in potential antioxidant compounds. However, the selenium concentration—which is known to function as an antioxidant to protect cells from oxidative stress (25)—was not provided. Using inductively coupled plasma mass spectrometry (ICP-MS) (26), we measured selenium levels (see the Supplementary Material) in each serum (Table 1). A large range of selenium concentrations was evident across the sera and even across batches of the same brand of serum. SS contained the highest levels of selenium, which was 3.5× higher than the Invitrogen FBS sourced from Australia [Inv-AUS (Cat. no. 10099; Invitrogen, Mulgrave, Australia)]. We then selected several sera that exhibited a range of selenium concentrations, to assess if they may generate differences in gene expression data.

MDA-MB-231 cells were grown in RPMI medium supplemented with five different sera, which are indicated by the codes provided in Table 1, and transfected with either a control plasmid (pGL3-Basic) or with the Trx gene promoter construct. By using a 10% level of serum, the
MDA-MB-231 cells were grown in final concentrations that ranged from 16.46 nM selenium for Inv-AUS FBS to 58.26 nM selenium for SS-cultured cells. The cells were stimulated with tBHQ for 20 h and luciferase assays performed (see the Supplementary Material). Expression levels were compared with nonstimulated cells and fold induction was determined. Transfection efficiencies were assessed by transfecting cells with pEGFP-C3 and were not significantly different when cells were grown with different sera in each of the experiments (data not shown). As shown in Figure 1A, cells grown in media supplemented with the serum containing the highest selenium level (SS) resulted in the lowest fold induction of the Trx gene promoter, while sera with the lowest levels [QS (Cat. no. A15–501; PAA, Murarrie, Australia)] and Inv-AUS resulted in the highest fold inductions. Cells grown in sera that contained intermediate selenium concentrations [Lonza (Cat. no. 14-501F, Lonza, Mt. Waverley, Australia) or Inv-US (Cat# 26140, Invitrogen)] exhibited a correspondingly intermediate induction fold. This suggests that cells grown in the serum-containing medium with the higher selenium concentrations have a greater capacity for resistance to oxidative stress and hence need less to stimulate the Trx gene promoter. Since cells grown in QS FBS yielded lower induction folds than those grown in Inv-AUS serum despite similar selenium concentrations, there may be additional serum components also involved in determining the cellular response to an oxidative stress—causing stimulus. However, the observed trends are overall consistent with the higher fold inductions occurring in cells grown with lower levels of selenium.

These results suggest that cells grown in medium supplemented with serum containing higher selenium concentrations will respond differently to stress-causing reagents than those grown using serum containing lower selenium concentrations.

Since many cell processes are under redox control through either redox-regulated transcription factors (9–14,18) or through redox-regulated cell-signaling pathways (7,27), many gene expression studies may be affected. We grew cells in medium supplemented with serum containing low and high levels of selenium (QS and SS, respectively) to determine if promoter activity of a nonantioxidant gene promoter may also be differentially stimulated. Hypoxia inducible factor-1 (HIF-1) is a widely studied transcription factor that is activated during hypoxic growth and binds to the hypoxic responsive element (HRE), which is present in the promoters of its target genes (28). Its activity is regulated by redox factor-1 (Ref-1), which functions to reduce a specific cysteine residue within the α subunit of HIF-1 (11). Reduction of this cysteine residue is necessary for HIF-1 to bind to co-activator molecules. Ref-1 in turn is regulated by Trx (12).

MDA-MB-231 cells were transfected with either a construct containing four copies of the HRE or with a control plasmid (pGL3-promoter). Cells were grown under either normoxic or hypoxic conditions before luciferase activity was measured. The results are shown in Figure 1B and clearly demonstrate that cells grown in medium supplemented with QS serum have the greater level of induction of the HRE construct when stimulated with hypoxia. Cells grown with SS, which contains higher selenium levels, display a lower level of induction. This result shows that serum selenium levels can also affect induction of a promoter construct regulated by HIF-1 in response to hypoxia. The selenium content of the serum does not solely influence the expression from antioxidant gene promoters but rather has consequences for expression from other gene promoters that may be activated by any of the many redox-regulated transcription factors, including HIF-1 (11), NF-xB (13), AP-1 (12), p53 (14) and Sp1 (10).

This change in promoter activity was not due to altered growth rates of the cells. MTT proliferation assays were performed on MDA-MB-231 breast cancer cells (Supplementary Figure S1) and proliferation was not significantly different when cells were grown in medium supplemented with either 10% FBS (Inv-AUS) or 10% SS.

The change in promoter activity is likely to be as a consequence of altered functionality of the cellular redox systems. Selenium is an essential trace element for growth as it is required for the catalytic activities of two key antioxidant enzymes, TrxR (29) and GPx (15). To determine the effects of serum selection on antioxidant enzyme activity in our cell line, we measured the activity of three antioxidant enzymes [TrxR, GPx, and a non–selenium-dependent enzyme glutathione reductase (GR)] (30) when cells were grown in medium supplemented with either SS or FBS (Inv-AUS). As shown in Figure 2, the specific activities of all three antioxidant enzymes were greater in the cells grown in SS, which had the higher selenium concentrations.

We also grew the cells in the same media supplemented with selenium over a range of 25–200 nM. As shown in Figure 2, the activities of TrxR and GPx were significantly increased in cells grown in FBS containing medium supplemented with 25 nM selenium; higher selenium concentrations produced no further increases. In contrast, when selenium was added to cells grown in medium containing SS, no increases in TrxR and GPx activities were observed, indicating that endogenous selenium levels in this serum were sufficient for optimal expression of these enzyme activities. In both the FBS- and SS-containing media, the cell’s GR activity levels were not responsive to selenium supplementation as might be expected for a non-selenoprotein. The increased enzyme activities
of TrxR and GPx levels. These increased TrxR activity will have effects on gene expression and redox-regulated promoters; the rate of expression and induction levels of these promoters when treated with various stimuli will therefore differ in cells grown in different serum. In this study, we utilized two different promoter constructs stimulated by two different stresses under the control of two different transcription factors (Nrf-2 and HIF-1). These transcription factors are each responsible for induction of several hundred genes (33,34) and thus regulate many important and well-studied pathways. The promoter constructs were both induced to higher levels by their respective stressors when cultured in medium supplemented with serum containing a lower selenium level, compared with when grown in higher selenium levels. Higher selenium levels result in more active antioxidant systems, as seen by increased TrxR and GPx activity, thereby resulting in a less oxidizing environment with less requirement to induce high-level expression of additional antioxidants such as Trx when subjected to more stress. Thus, other genes induced by thioredoxin-regulated transcription factors may also be less highly stimulated. Due to its importance in determining the redox state of the cells, selenium concentration is an important metric that companies should provide, as it can influence data obtained and reported by different groups. The provision of such information will help researchers determine whether experimental outcomes can be attributed to differences in chemical composition of sera that may occur across brands and even between batches of the same brand.

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Competing interests
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References

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