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Proteomics analysis of altered cellular metabolism induced by insufficient copper level

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ABSTRACT

Insufficient copper level in the mammalian cell culture medium resulted in lactate accumulation while maintaining similar growth and culture viability profiles. Label-free, LC-MS/MS-based shotgun proteomics method was applied to compare the protein expression profiles obtained from the cultures exposed to suboptimal copper level to those provided with sufficient amount of copper. Under copper deficient condition, a substantial reduction of the protein levels of the multiple subunits of Complex IV, also known as cytochrome c oxidase, of the mitochondrial electron transport chain was observed for all three different Chinese Hamster Ovary (CHO) cell lines expressing therapeutic monoclonal antibodies tested. Additional proteins affected by suboptimal copper level included peroxiredoxin (PRDX) and hepatocyte-derived growth factor (HDGF), which were affected during early phase of the fed-batch production, several days prior to initiation of lactate accumulation. In contrast, proteins such as syntenin (SDCBP) and integral membrane 2C (ITM2C) showed altered expression patterns toward the end of culture duration, after lactate divergence had occurred. For all conditions tested, time was the most predominant factor facilitating the direction of global protein expression trend, with substantial number of proteins subjected to time-dependent changes in expression, independent of copper.

1. Introduction

Copper is an essential trace element which functions as a catalytic and structural cofactor for various enzymes (Olivares and Uauy, 1996; Turski and Thiele, 2009; Xu et al., 2013). Menkes and Wilson's diseases are the inherited disorders of copper metabolism in humans which result in systemic copper deficiency and accumulation, respectively (de Bie et al., 2007). Patients of Menkes disease have mutations in the *ATP7A* gene encoding for Copper-Transporting P-type ATPase 1 (Polishchuk and Lutsenko, 2013; Tumer and Moller, 2010), and they are affected with multiple symptoms including growth retardation, progressive neurodegeneration, connective tissue defects, hypothermia and hypopigmentation. These pleiotropic symptoms are presumed to be the result of multiple copper-dependent enzymes malfunctioning due to copper deficiency mediated by malabsorption of dietary copper (Menkes et al., 1962; Tumer and Moller, 2010). Interestingly, elevated levels of lactate have been detected in the brain and the blood of Menkes patients (Loyola and Dodson, 1981; Munakata et al., 2005; Rizzo et al., 2000), implicating a functional link between copper and lactate. Similar to Menkes patients, augmented brain and blood lactate levels were found in copper deficient rodent models (Gybina and Prohaska, 2009). In addition, higher tumor lactate levels were detected in mice treated with the copper chelator tetrathiomolybdate (TM) during tumor progression (Ishida et al., 2013).

Similar to elevated blood lactate levels detected in Menkes patients and copper deficient animal and tumor models, extracellular lactate accumulation was observed when insufficient amount of copper was provided to the recombinant Chinese hamster ovary (CHO) cells (Luo et al., 2012; Qian et al., 2011; Yuk et al., 2014). Since lactate increase is often accompanied by decrease in productivity and altered product quality, substantial efforts over the years have been devoted to understand and control lactate increase during industrial cell culture process (Korke et al., 2004; Li et al., 2010; Li et al., 2012; Mulukutla et al., 2012; Pascoe et al., 2007; Seow et al.,

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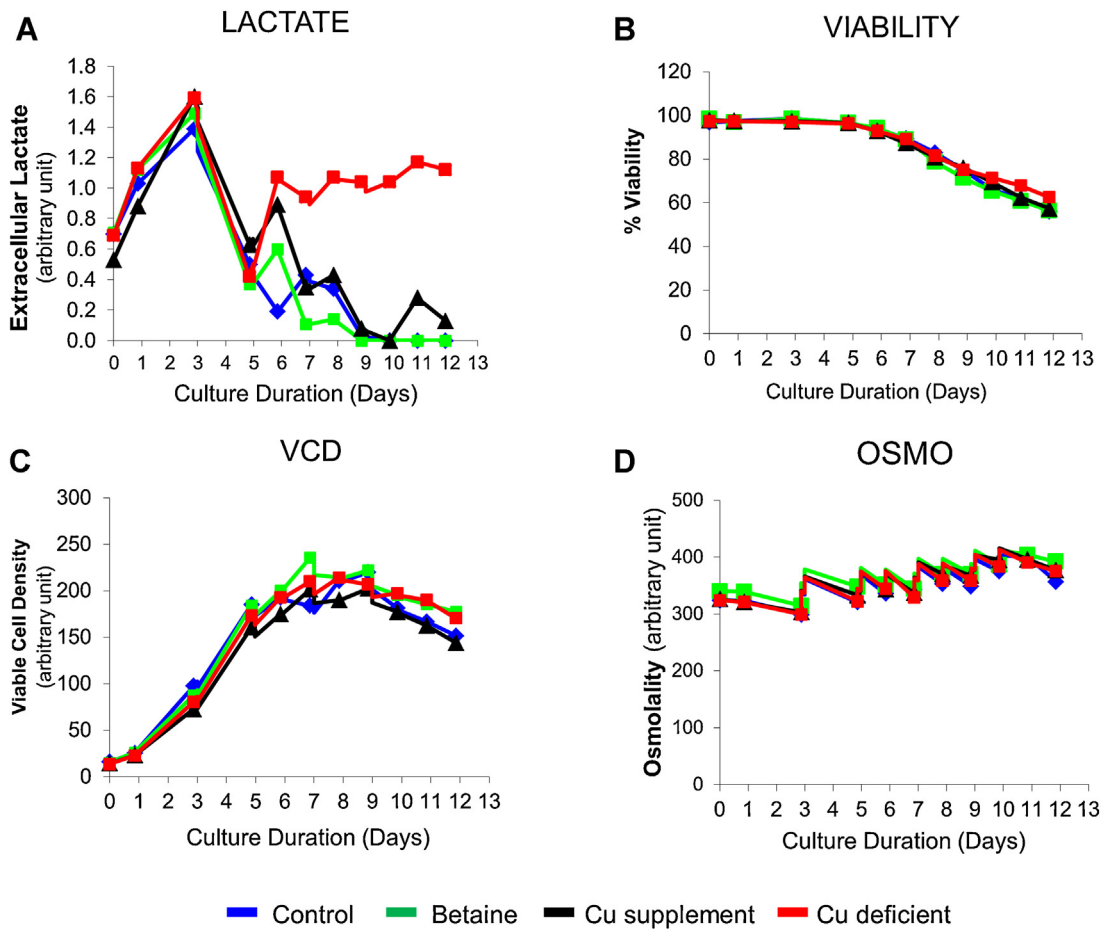

Fig. 1. Cell culture profile comparison between copper-sufficient vs. copper-depleted conditions in Cell Line A. (A) Extracellular lactate, (B) Culture Viability (%), (C) Viable Cell Density (VCD), (D) Osmolality.

2001; Zagari et al., 2013). Transcriptomics and metabolomics analyses have previously been performed to gain insight into cellular mechanisms associated with lactate accumulation and associated phenotypic changes induced by copper deficiency (Luo et al., 2012; Qian et al., 2011; Yuk et al., 2014).

In this study, we utilized the label-free, LC-MS/MS-based shotgun proteomics approach to investigate the molecular mechanisms and intracellular events associated with lactate accumulation induced by copper deficiency during fed-batch production of three different CHO cell lines. By directly comparing the protein expression profiles obtained from copper-deficient versus copper-adequate conditions throughout different time points during culture duration, we were able to identify proteins and pathways associated with copper deficiency.

2. Materials and methods

2.1. Cell Culture

A bioreactor experiment examining the performance of Cell Line A under copper-sufficient vs. copper-deficient conditions were performed by exposing the cell line to four different base production media on the inoculation day (i.e., Day 0). Medium powder #1 which contains the proprietary amount of copper sulfate was used for both the "Control" and "Betaine" conditions described in Fig. 1, with proprietary amount of betaine added to the medium used for the "Betaine" condition. For "Cu supplement" and "Cu deficient" conditions, medium powder #2, which contains the same media components and concentrations as medium powder #1 with the exception of copper sulfate which was completely removed from the medium powder, was used. For "Cu supplement" condition, the proprietary amount of copper sulfate was added back to the final medium prepared with medium powder #2, resulting in the same final concentration of $CuSO_4$ as the "Control" condition. For "Cu deficient" condition, no copper sulfate was added back. The four 2-L bioreactors, each representing one of the above described conditions, were fed with the same feed medium which contains the proprietary amount of copper sulfate on days 3, 5, 7 and 9. For Cell Line B and C, the cultures were treated with either Medium #3 which contains "high" or "medium" level of copper sulfate, or with Medium #4 which contains "low" amount of copper sulfate. A bolus feed was introduced on Days 3, 6 and 8. For both cell line A and cell lines B/C, a typical fed-batch production process was utilized with two-sided pH control. However, the culture medium composition and the amount of feed medium and feed schedules were different for cell line A *versus* cell lines B and C. The same process was applied to cell lines B and C.

2.2. Assays

Small volumes of culture were taken to assess viable cell density (VCD) and cell viability using the Cedex AS20 cell counter (Roche Innovatis, Beilefed, Germany) on days 1,3, and 5 through 12 during the fed-batch production run,. Metabolic data, including lactate, were obtained from the Nova Bioprofile 100 Plus (Nova Biomedical, Waltham, MA), and an Advanced Instruments