Using Genomic Tools for the Identification of Important Signaling Pathways in Order to Facilitate Cell Culture Medium Development

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Abstract: Given the diversity of cells grown in culture and the fact that each of these cultures is poised to respond to a variety of stimuli, we set out to establish a method to identify these important pathways to accelerate the development of cell culture media. Many of the pathways required for cell functions (i.e. cell proliferation, protein production, cell adhesion, etc.) have been elucidated in detail, but there are undoubtedly unknown pathways, which may also be involved. Identification of these resources allows us to better understand these processes and potentially manipulate them to our advantage.

In order to validate this concept, we identified the mRNA expression profile for a variety of proteins within cells in culture using cDNA microarrays. In one example, we identified a specific growth factor receptor not previously known to be expressed *in vitro*. When the ligand for this receptor is added to the culture, the cells are poised to respond and proliferate at an increased rate. This method has been used on a variety of culture systems to either reduce (or eliminate) FBS requirements or to improve the performance of already serum-free formulations. This more targeted approach to medium development allows us to perform less of the random screening approaches of the past, thereby decreasing the investment of time and resources into this endeavor.

Key words: Cell culture, medium, microarray, cell signaling, genomic tools, growth factors, receptors, CHO.

1. INTRODUCTION

One of the biggest concerns for the development of new cell culture media is the investment of time required to optimize these products for the

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intended culture. It is not atypical for the development of a new cell culture product to take more than one year. We have tried to provide a more targeted/focused approach to the design of cell culture products by using rational methods of identifying candidates for inclusion in a medium.

The influx of genomic and proteomic research into the scientific community has led to an increased number of tools that can be applied to the development of cell culture products. These tools enable the rational design of more robust media formulations by providing insights into the stimuli the cells are poised to respond to *in vitro*. These methods could include genomic tools such as microarrays and quantitative PCR or proteomic tools such as antibody-based arrays. These approaches allow us to look either at mRNA or protein levels within cells in culture and predict based on the expression patterns what might elicit a response. The expression patterns of receptors (for growth factors, cytokines, etc.), adhesion molecules, or cell signaling components indicate important pathways for us to explore. These pathways could have beneficial effects on a wide range of functions, such as regulation of proliferation, apoptosis, differentiation, adhesion, or production.

2. RESULTS AND DISCUSSION

In order to test whether or not we could use information about the expression profile of a cell culture to determine factors that the cells would respond to, we examined the profile of many growth factor/cytokine receptors in several different cell lines and/or culture conditions. We were able to identify lists of receptors that were expressed within the different cultures (see Table 1 below for a list of receptors from a CHO cell line expressing alkaline phosphatase (CHO-AP)). Upon addition of the ligands for these receptors (or alternatively endogenous intermediates for the pathways), we were able to manipulate cellular functions such as proliferation, productivity and adhesion. Figure 1 demonstrates this effect with the addition of interleukin 1 to CHO-AP, leading to both increased proliferation and productivity of the recombinant protein. This methodology not only allows us to improve on existing serum-free cell culture medium formulations, but also allows us to reduce or eliminate FBS from FBS-dependent cultures.

The use of genomic or proteomic tools appears to be a powerful tool for use in medium development, allowing us to speed development time and correspondingly reduce costs. Based on the accumulation of data from various cell lines, we can apply a variety of development tools (microarray, macroarray, qPCR, antibody arrays, etc.) to cells in culture in order to enhance our ability to quickly design cell culture media. These tools allow us

to provide a more targeted approach that is both reproducible, with higher throughput and will have a significant impact on the development time.

Table 1. CHO-AP: List of positive receptor:ligand pairs identified from microarray.

Receptor	Ligand	Receptor	Ligand
Interleukin 12 receptor, β 2	IL-12	TGF, β receptor II	TGFβ
CSF 1 receptor	CSF1	Activin A receptor, type II	Activin A
Burkitt lymphoma receptor 1	BLC	Macrophage stimulating 1 receptor	MSP
Chemokine receptor 9	TECK	Interferon γ receptor 2	IFNγ
Interleukin 11 receptor, α	IL-11	BMP receptor, type II	BMP2
BMP receptor, type IA	BMP2	G protein-coupled receptor 9	MIG
FGF receptor 4	aFGF	Activin A receptor, type I	Activin A
Interleukin 1 receptor-like 1	IL-1	FGF receptor 1	bFGF
Chemokine receptor 4	MDC	IGF 2 receptor	IGF2
Chemokine receptor 1	MCP3	Autocrine motility factor receptor	PGI
PDGF receptor, β polypeptide	PDGF AB	GDNF family receptor α 3	Artemin

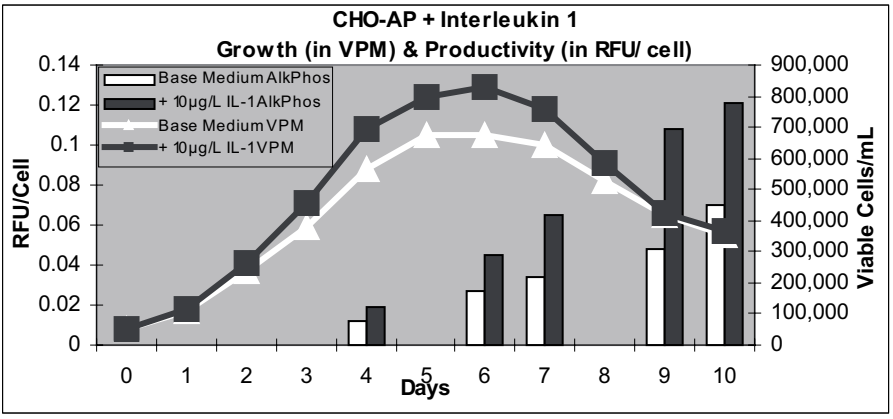

Figure 1. Growth and productivity of CHO-AP cells in spinner culture is enhanced with the addition of interleukin 1.