Cost-effective and Scalable Production of Authentic Recombinant Human Proteins from Human Cells

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Cost-effective and Scalable Production of Authentic Recombinant Human Proteins from Human Cells

Recombinant proteins (r-proteins) have been increasingly important in fundamental research as well as for preclinical and clinical applications. Because many of these r-proteins are of human origin, cultivated human cells are the optimal choice to ensure authentic biophysical properties and functional activities, which depend on proper folding, phosphorylation, disulfide bridge formation, proteolytic processing, and glycosylation (Table 1). However, the current expression systems are inefficient, and it is often a daunting task to produce a sufficient amount of quality proteins (1-100 mg) at an acceptable cost. HumanZyme has developed a high yield and cost-effective versatile system, HumaXpress™, to produce active recombinant human proteins in a human HEK derivative cell line in serum-free and chemically defined media. Using this technology, our scientists have successfully produced a large number of active and authentic human cytokine and kinase reagents designed to meet the special needs of the broad scientific community and biotechnology industry. Comparative studies indicate that the recombinant human proteins from human cells are superior to those produced in non-human cell systems.

TABLE 1

Expression System	E. coli	Insect Cell	Cho Cell	Human Cell
Protein Folding	●○○○	●●○○	●●●○	●●●●
Phosphorylation	○○○○	●●○○	●●●○	●●●●
Proteolytic Processing	○○○○	●○○○	●●●○	●●●●
Glycosylation	○○○○	●○○○	Not Human-Like	●●●●

Recombinant Human Cytokines

Most human cytokines are glycoproteins less than 30 kD in size. Due to their central role in the immune system, cytokines are involved in a variety of immunological, inflammatory and infectious diseases and widely used in research, diagnostics and therapeutics. Currently, these proteins are predominantly produced in non-human cell expression systems (e.g. E coli, SF9 or CHO) and therefore lack authenticity due to the absence of physiologically relevant glycosylation. In addition, a number of important cytokines such as those belonging to the TGF-ß superfamily are difficult to produce in sufficient quantity due to inadequate proteolytic processing, protein folding or other post-translational modifications that occur in the non-human cell expression systems. Our work with VEGF165 and IL-4 demonstrates that the recombinant cytokines from human cells are differentiated from the non-human cell version and can be used as preferred reagents for research and antibody development.

VEGF165 plays a prominent role in normal and pathological angiogenesis. It has been demonstrated that inhibition of VEGF activity by treatment with a monoclonal antibody specific for VEGF can suppress tumor growth in vivo. Currently,

commercially available VEGF165 protein reagents are produced from non-human cells including E coli and insect cells. HumanZyme has produced VEGF165^{HuXp} from human cells adapted to chemically defined media. As shown in Fig. 1a, the molecular mass of the E. coli expressed protein in monomer is 18 kD. This compares with the VEGF165^{HuXp} which migrates as a band of 28kD due to glycosylation. The bioactivity of VEGF165 was determined by its ability to induce proliferation of human umbilical vein endothelial cells, indicating VEGF165^{HuXp} is 6-fold more active than the E coli expressed protein (Fig. 2a). IL-4 plays a critical role in the development of allergic inflammation and asthma. Currently, commercially available IL-4 protein reagents are produced from E. coli with a molecular mass of 14 kD (Fig. 1b). This compares with the IL-4^{HuXp} from human cells which migrates as a major band of 19 kD due to glycosylation. The biological activity of IL-4 was determined by the dose-dependent stimulation of the proliferation of human TF-1 cells. As shown in Fig. 2b, IL-4^{HuXp} has 4-fold higher potency than the E. coli expressed cytokine. (Fig. 2b)

FIGURE 1

a and c: SDS PAGE stained with Coomassie blue
b and d: Western blot analysis
1 and 3: under non-reducing conditions
2 and 4: under reducing conditions

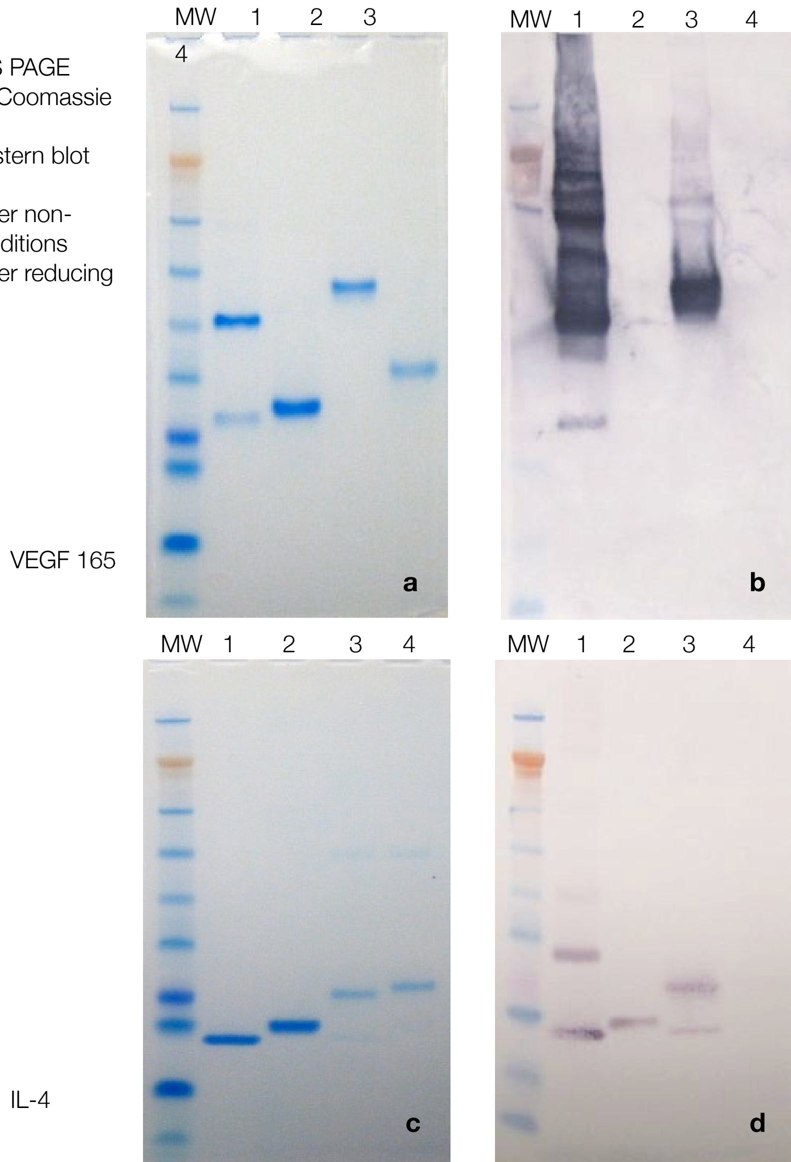


FIGURE 2a

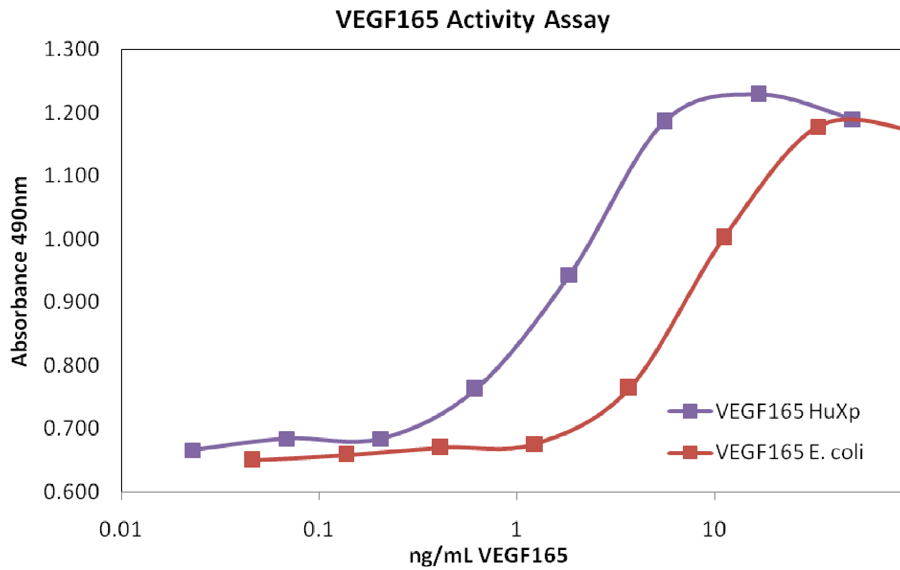
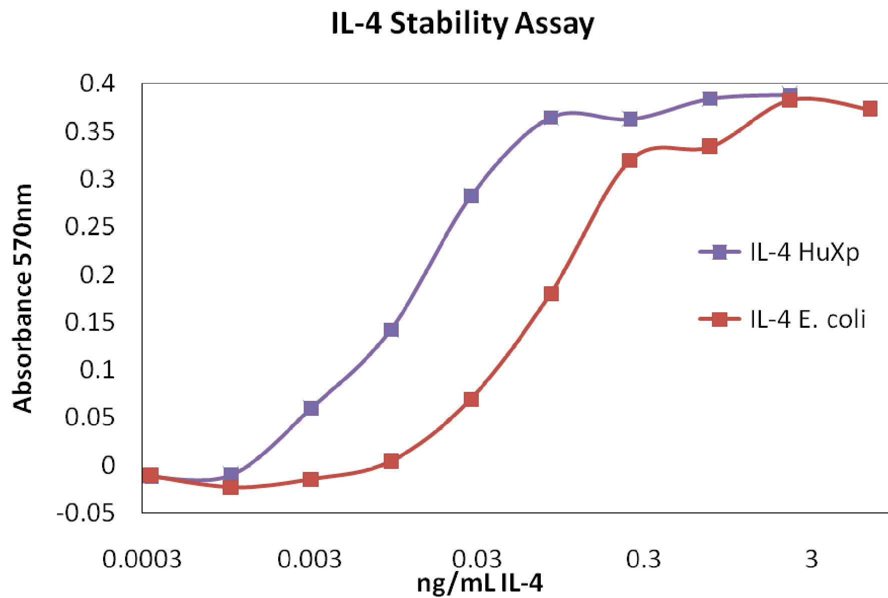


FIGURE 2b



Cytokines produced in E coli are not glycosylated and may expose cryptic or normally hidden epitopes. Hence, antibodies may have different affinities for native human proteins compared to the E coli produced proteins. Indeed, Western blot analysis shows the monoclonal antibodies raised against a full length protein from insect cells recognize the VEGF165 protein from E coli as well as other highly reactive species that may correspond to micro-aggregates (Fig. 1c). In contrast, only one band is seen with the human cell version. The monoclonal antibodies raised against a full length

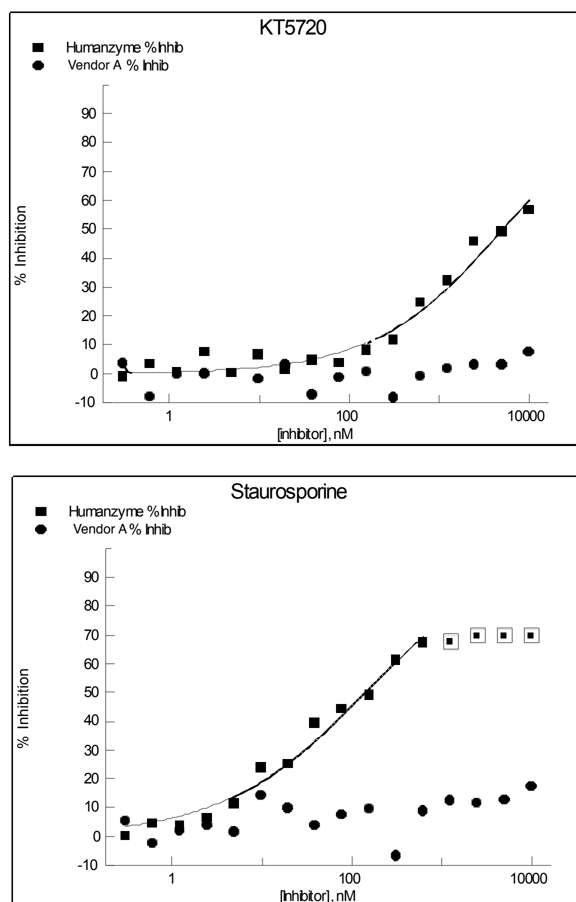
protein from E coli recognize the IL-4 protein from E coli under both reducing and non-reducing conditions (Fig. 1d). In contrast, only the protein under non-reducing conditions is detected with the human cell version.

Recombinant Human Protein Kinases

Due to their critical role in intracellular communication, dysregulation of protein kinases has been implicated in as many as 400 human diseases including cancer, diabetes, heart disease, neurological disorders and rheumatoid arthritis. Hence, protein kinases are important for drug design and screening. Currently, kinases are predominantly produced in non-human cells (e.g. E coli or insect cells) many of which require protein truncation and/or in vitro activation steps, due to the limitations of the expression system. HumanZyme has expressed more than 100 recombinant human protein kinases which are full length and in vivo activated including difficult to produce ATM and mTOR kinases. Using p38α as an example, we have demonstrated that the properties and inhibition profiles of the human protein kinases produced in human cells are differentiated from versions of the same kinase that were produced in non-human cell systems. This will allow researchers to avoid pursuing false negative leads and missing promising targets.

$p38\alpha^{HuXp}$ was produced and activated in human cells in the presence of arsenite. Sample kinases from Vendor A and B were expressed and purified from E. coli, in vitro activated by MKK6, and repurified. SDS PAGE analysis shows that p38α produced in the human cell expression system is pure with a dominant band of 60 kD and minor band of endogenous human GST of 23 kD. This was confirmed by MS analysis and no other contaminant proteins were found. The $K_{m.ATP}$ for $p38\alpha^{HuXp}$ is 109 ± 12 μM while the K_m was 212 ± 26 μM for the Vendor A preparation. The K_m of 120 μM was found with Vendor B enzyme. The IC_{50} values were determined for 14 known kinase inhibitors (Table 2). While the IC_{50} values for SB-202190 (the known p38α selective inhibitor) for both p38α preparations were similar (0.02 μM and 0.03 μM respectively), there is clearly a difference in the sensitivity to the inhibitors between $p38\alpha^{HuXp}$ and Vendor A preparations (Fig. 3; Table 2).

FIGURE 3



The Vendor A preparation was only sensitive to AMP-PNP (a non-hydrolysable ATP analog). Yet, the protein was 7-fold less sensitive than p38α[HuXp], which is consistent with its higher K_m. p38α[HuXp] on the other hand, had measurable IC_{50} values against staurosporine, K252a, Ro 31-8220, KT5720, and SB-202190. The inhibition profile of Vendor A kinase is comparable to that of Vendor B (Table 2).

TABLE 2. Inhibitor IC50 Values (IC50 μM)

	Humanzyme	Vendor A	Vendor B
Staurosporine	0.14	>10	>10
H-9 dihydrochloride	>10	>10	>10
AMP-PNP	257	1806	>2000
HA-1077 dihydrochloride	>10	>10	>10
Rotterline	>200	>200	>200
H89 dihydrochloride	>10	>10	>10
5-iodotubercidin	>10	>10	>10
K252a	0.005	>10	>10
Ro 32-0432	>10	>10	>10
Ro-31-8220	9.9	>10	>10
GF 109203X	>10	>10	>10
KT5720	5	>10	>10
Imatmib mesylate	>10	>10	>10
SB	0.02	0.03	0.01

Taken together, the current study demonstrates that the properties of the human cytokines and protein kinases produced in human cells are distinct from those produced in non-human cell systems. Ever since Genentech scientists produced the first recombinant human protein in E coli in 1977, recombinant protein expression in heterologous hosts has played a critical role in the launch of the entire modern biotechnology industry. With the widespread availability of authentic and cost-effective human protein reagents from human cells, we are now at the point of a paradigm shift, where authentic proteins will be widely used as the preferred research and diagnostic reagents, as well as for drug screening and antibody development, thereby greatly improving the speed and quality of both basic research and pharmaceutical development.