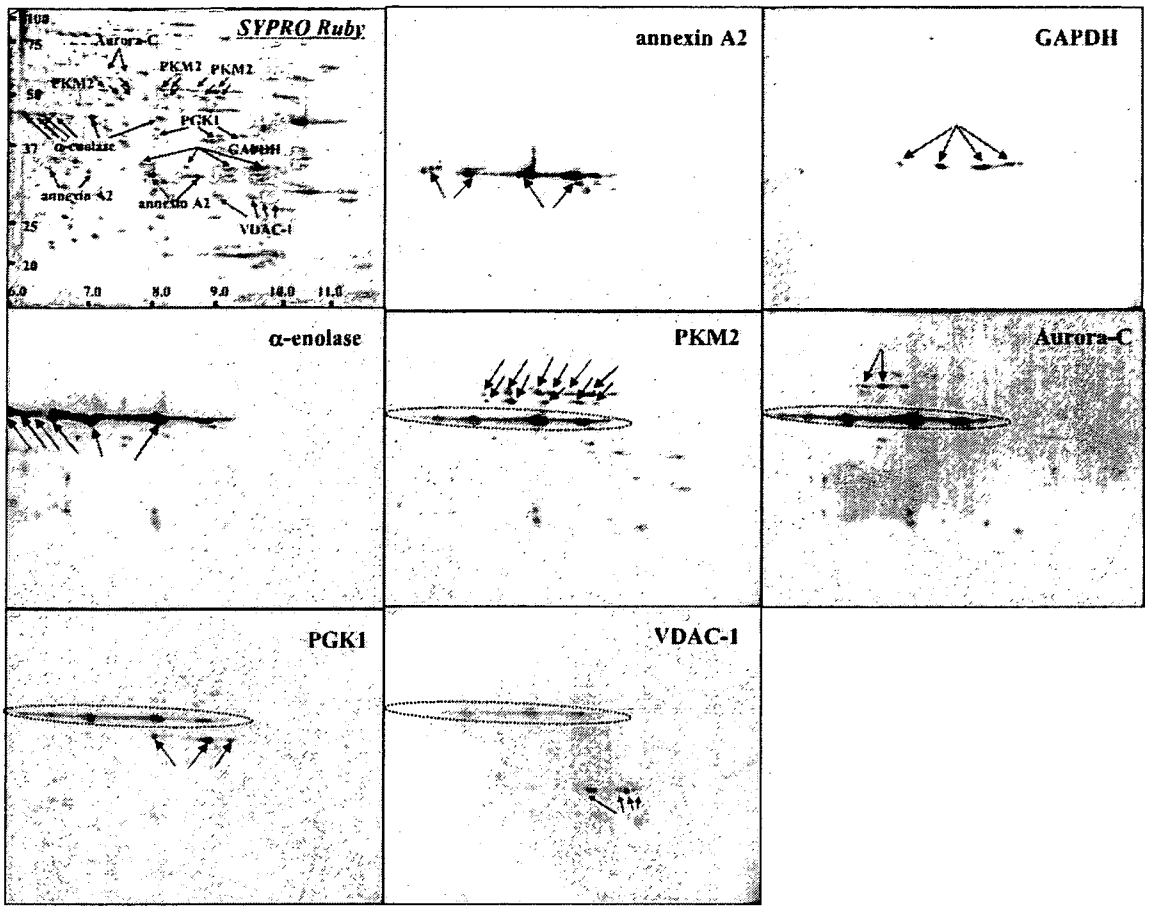

Fig. 3. Two dimensional western blotting after a pH 6–11 strip-using 2-DE gel.

The 2-DE gel was trimmed (an upper left panel) and transferred to the PVDF membrane. Western blotting was performed using indicated polyclonal antibodies, which are shown to have broad cross-reactivity among human, mouse and rat by the manufacture. The PVDF membrane was re-used after stripping the previously used antibody. The multiple spots and slurs in an area surrounded by a dotted line in PKM2, Aurora-C, PGK1 and VDAC-1 antibody reactions are the background spots stubbornly remained after the α-enolase antibody reaction.

human databases did with lower % coverage values (data not shown).

The results of the 2D western blotting further revealed that some of the proteins were expressed as multiple spots with similar molecular weights but different pI values, indicating that those proteins have multiple isoforms via modification such as phosphorylation and acetylation. Our study, in which a combined analysis of western blotting and mass spectrometry was performed, could identify more protein isoforms in monkey ES cells than a report did on mouse ES cells, where a sole mass spectrometry analysis was performed (Table 3). Although most of the proteins identified in monkey ES cells were also detected in mouse ES cells[10], we found that some proteins were unique to the monkey ES cells. For example, annexin A family proteins were not detected in mouse ES cells, while annexins A2, A5 and A8 were clearly detected in monkey ES cells. Because the 2-DE proteomic study on mouse ES cells[10] was extensively performed by mass spectrometry successfully identifying as many as 123 protein spots in pH 3–10 strip-using gel, it seems that annexin A family proteins are not expressed, or if any, in undifferentiated mouse ES cells. Other examples are Aurora-C and pigment epithelium-derived factor, whose murine homologues have not been reported so far. We conclude that the human proteome databases, consisting of a large volume of information with high quality organization, are of a significantly great service in identifying monkey proteins.

We showed here a draft protein expression profile of undifferentiated cynomolgus monkey ES cells by 2-DE proteomic studies. We have successfully identified multiple protein spots via a combined analysis of mass spectrometry using human databases and western blotting using polyclonal antibodies. We also found that non-human primate databases are not so useful as human databases in identifying monkey proteins. This finding coincides with a previous report, which is the only one report that has ever reported on the 2-DE-based proteomic analysis using monkey samples[11]. In this report, heart samples of individual cynomolgus monkeys were subjected to 2-DE, and the protein

Table 3. The protein isoform detected in monkey and mouse ES cells

protein name	cynomolgus monkey	mouse
elongation factor 2	3	3
glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	4	1
pyruvate kinase isozymes M2 (PKM2)	10	2
α-enolase	7	3
voltage-dependent anion-selective channel protein 1 (VDAC-1)	4	1
serine/threonine-protein kinase 13 (Aurora-C)	2	0
annexin A2	5	0

The number of the multiple spots detected by a combined study of mass spectrometry and western blotting in cynomolgus monkey ES cells (present study) and those detected by mass spectrometry in mouse ES cells reported elsewhere (reference No. 10) was listed.

identification was achieved by a MALDI-TOF-mediated mass spectrometry querying human databases. Thus, human databases are substantially available for identifying monkey protein spots in 2-DE.

By virtue of 2-DE western blotting analysis, we could identify multiple isoforms of common cytoplasmic proteins including enzymes involved in glycogenesis/glycolysis pathways such as GAPDH, α-enolase and PKM2. Among these, GAPDH is recognized as a multi-function protein, playing roles in endocytosis, microtubule bunding, phosphotrasnferase, nuclear RNA transport, DNA replication, DNA repair, vial pathogenesis, oncogenesis and apoptosis[12]. The multiple isoforms we identified in monkey ES cells might be related to its multiple functions. Other enzymes shown here to have multiple isoforms may also have multi-functions other than glycolytic regulation.

In conclusion, 2-DE–based proteomic studies using monkey samples can sufficiently be achieved via a combined analysis of mass spectrometry querying human databases and western blotting using polyclonal antibodies raised against human epitopes.

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