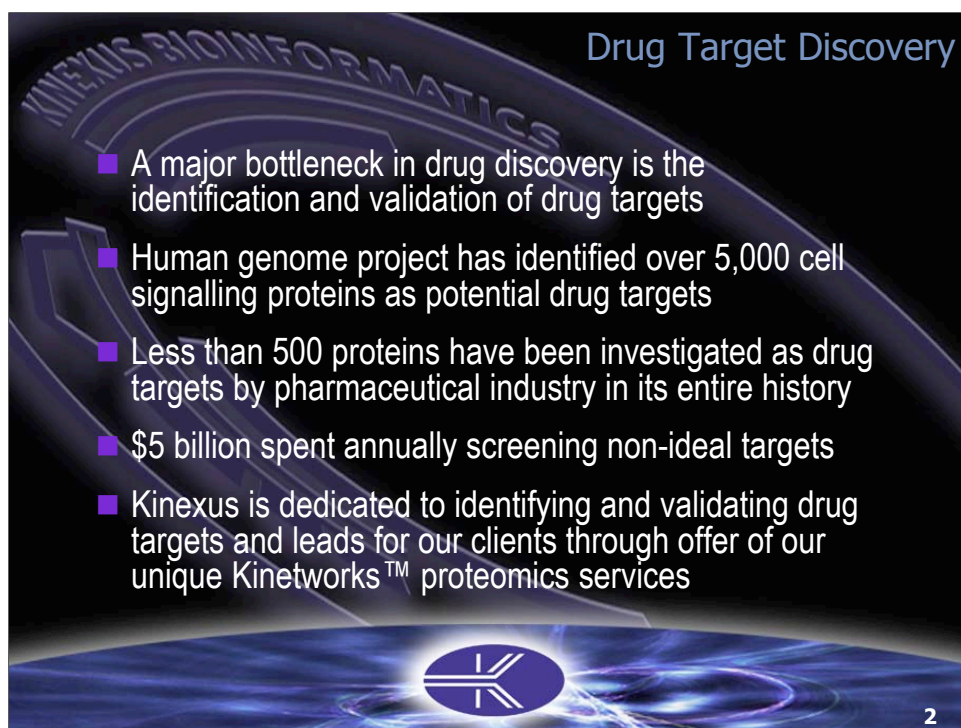




This presentation demonstrated the power of the Kinetixworks™ approach to uncover significant research results in a cost effective and efficient manner.



Drug Target Discovery

- A major bottleneck in drug discovery is the identification and validation of drug targets
- Human genome project has identified over 5,000 cell signalling proteins as potential drug targets
- Less than 500 proteins have been investigated as drug targets by pharmaceutical industry in its entire history
- \$5 billion spent annually screening non-ideal targets
- Kinexus is dedicated to identifying and validating drug targets and leads for our clients through offer of our unique Kinetworks™ proteomics services

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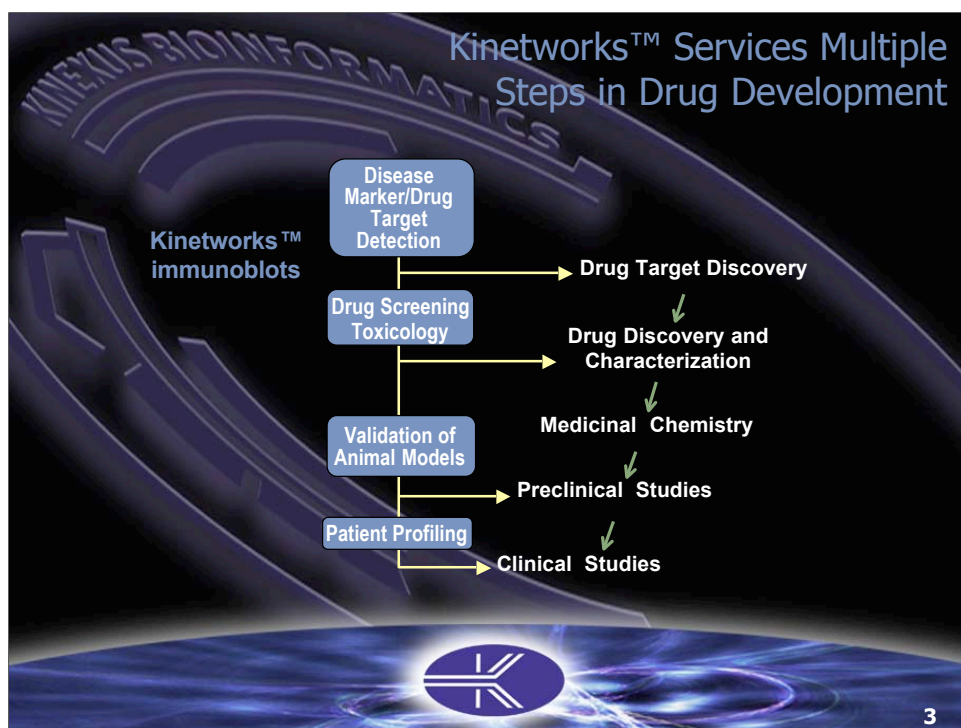
Drug discovery is a very risky and expensive proposition. Only one in a hundred thousand compounds may become a successful drug, after the investment of over US \$600 million on average and frequently more than 10 years in development.

To discover a drug, the right target must be identified first. This target can be used as the bait to fish for small molecule compounds that are typically inhibitors of the chosen target by high throughput screening. Lead compounds are put sequentially through cell-based, then animal-based and finally human trials. Each subsequent step of the drug discovery and validation process is associated with dramatic increases in cost. Only about 1 in 10 lead compounds that look promising at the end of the animal trials make it successfully through Phase III human trials and receive FDA approval. It is this high rate of failure at the late stages of clinical trials that has made drug discovery so expensive. If the failures could be identified earlier in the drug discovery process, this would markedly reduce costs and lost opportunity.

A common cause of failure in human trials is adverse drug reactions. The Kinexus Kinetworks™ screening platform for cell cycle, stress and apoptosis proteins can help to identify at an early stage possible toxic drug reactions and prevent the investment of millions of dollars on the testing of non-ideal targets.

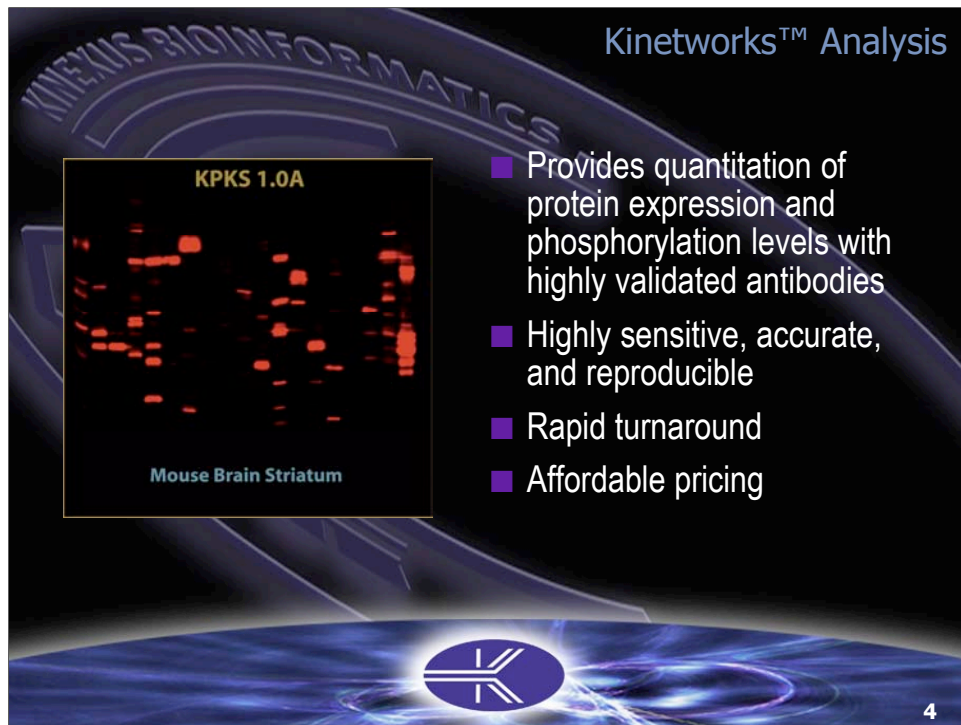
To have the greatest impact on drug discovery, it is critical to identify the most appropriate drug targets. This is a significant issue, because over \$5 billion is spent annually by the pharmaceutical industry screening against targets that even if specific, potent and cell permeable inhibitors were uncovered, the side effects associated with knocking out these targets would mitigate their utility.

With the identification of over 30,000 genes in the human genome, about 5000 cell signalling proteins have been designated as potential drug targets. However, the pharmaceutical industry does not have the capacity to evaluate all of these proteins for use in high throughput drug screening. In fact, less than 500 proteins have been examined extensively as potential drug targets by the pharmaceutical industry over the last century.



An important outcome of the Kinetworks™ proteomics services offered by Kinexus is the generation of vast amounts of data about the regulation of specific proteins. This unique immunoblotting service is only one of many novel services that Kinexus plans to offer its clients.

The data generated from the Kinetworks™ services is being merged into our Kininformatics™ functional proteomics database, which we are mining to establish the composition and architecture of protein kinase signalling networks in hundreds of different normal and pathological tissues and cells from diverse species. Our clients will also have Internet access to this database by subscription to KiNET™ bioinformatics services. The central objective of our endeavors is to identify protein kinase signalling pathways that link to specific diseases to identify diagnostic markers and protein kinase targets for drug discovery. Clients are able to query KiNET on-line about the expression patterns and phosphorylation states of hundreds of signalling proteins in hundreds of different model systems.



Kinetworks™ Analysis

KPKS 1.0A

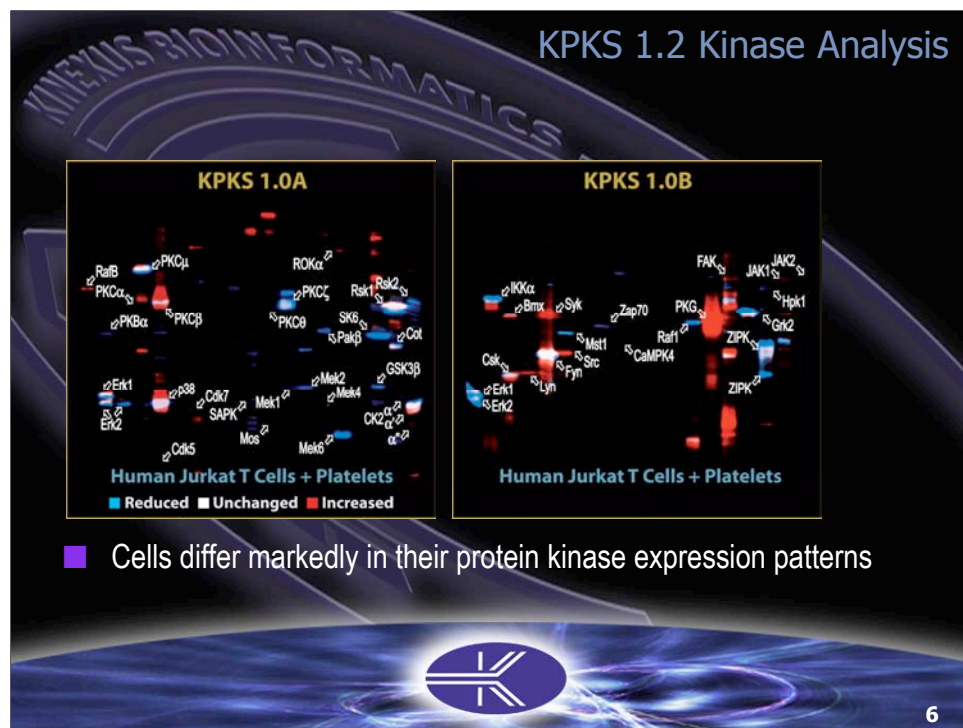
Mouse Brain Striatum

- Provides quantitation of protein expression and phosphorylation levels with highly validated antibodies
- Highly sensitive, accurate, and reproducible
- Rapid turnaround
- Affordable pricing

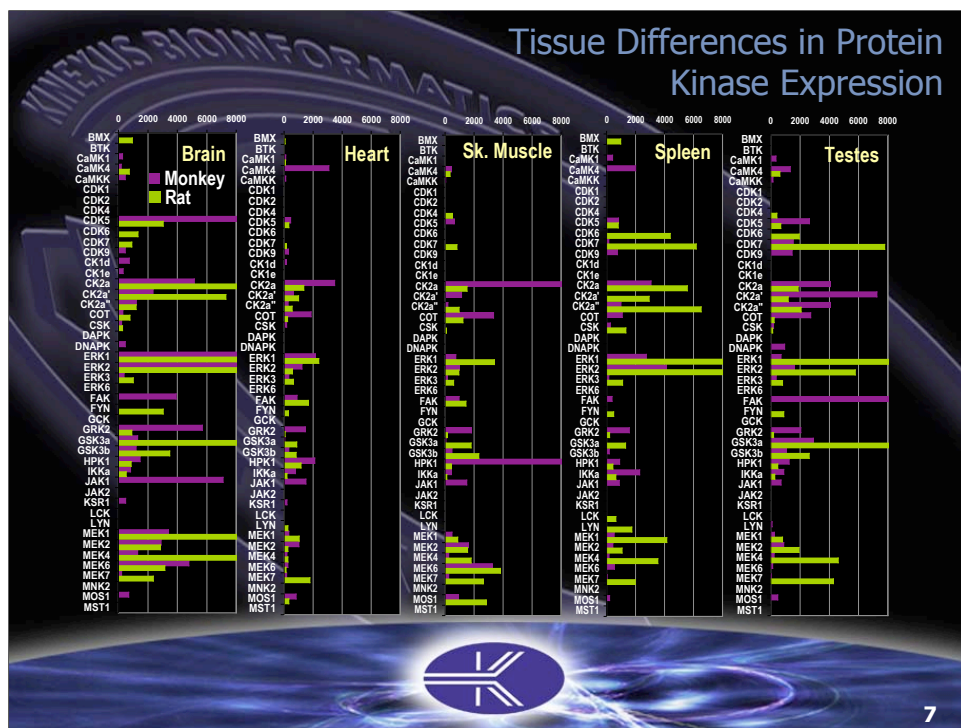
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The Kinetworks™ analysis utilizes commercial antibodies that have been highly validated by Kinexus to ensure accuracy. Enhanced chemoluminescence and densitometric analysis with a FluorS Max Imager from Bio-Rad permits sensitive detection with as little as 350 µg of lysate cell/tissue protein. Data is linear over a 2000-fold range with standard deviations within 20% for high reproducibility. Results are returned to clients within 4 weeks. Substantial discounts are available with full disclosure of sample information or bulk orders.

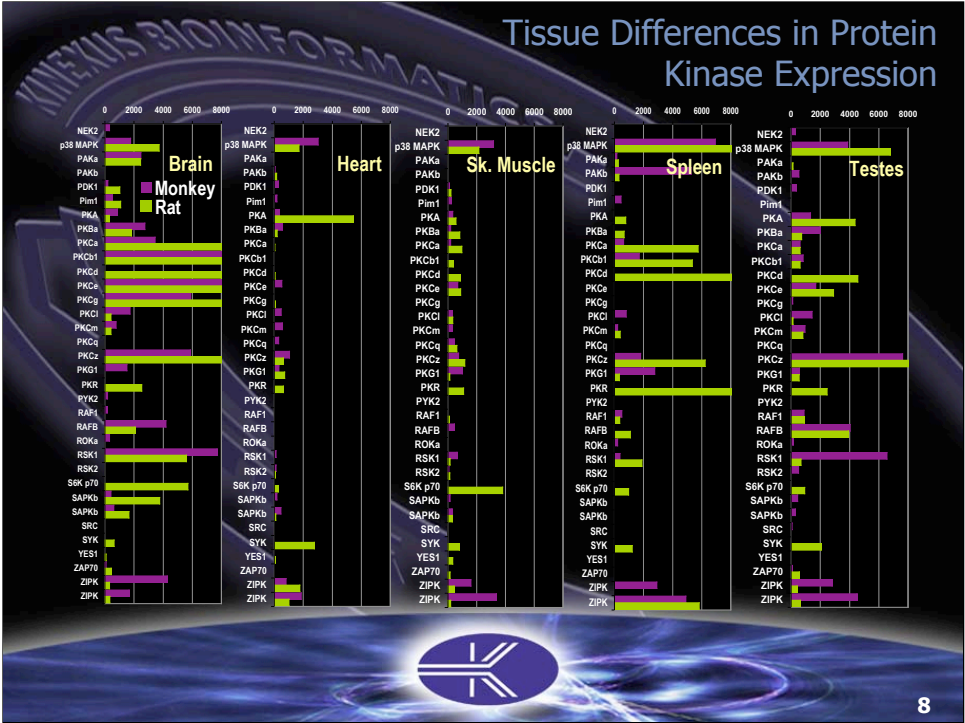
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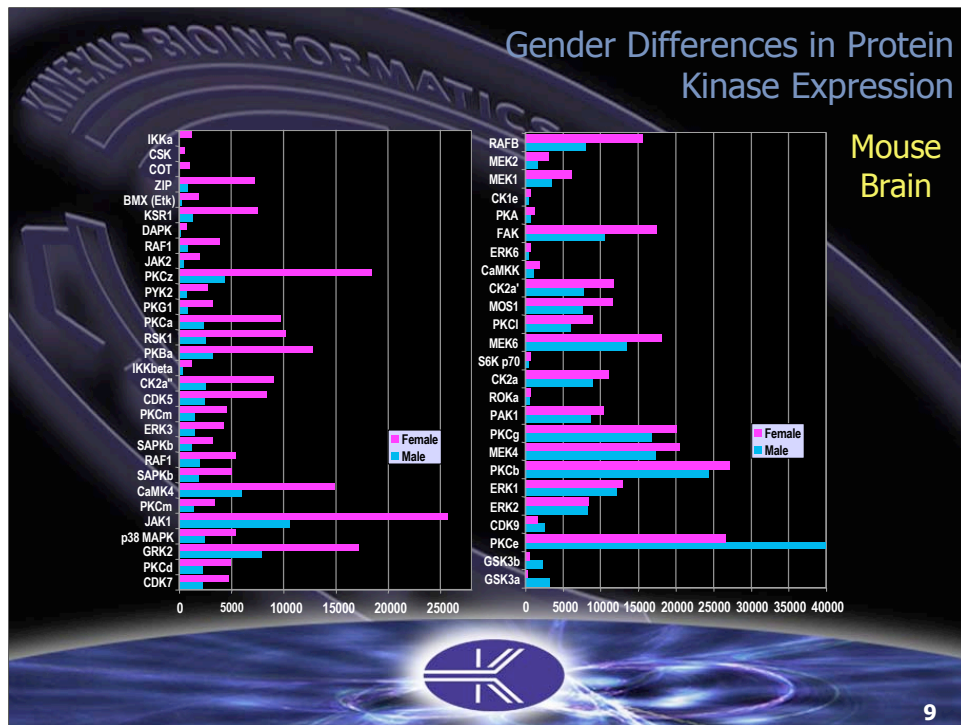


Kinexus has performed over 10,000 immunoblot analyses. These studies have revealed the vast differences between cell types in the expression patterns of protein kinases and other signalling proteins.

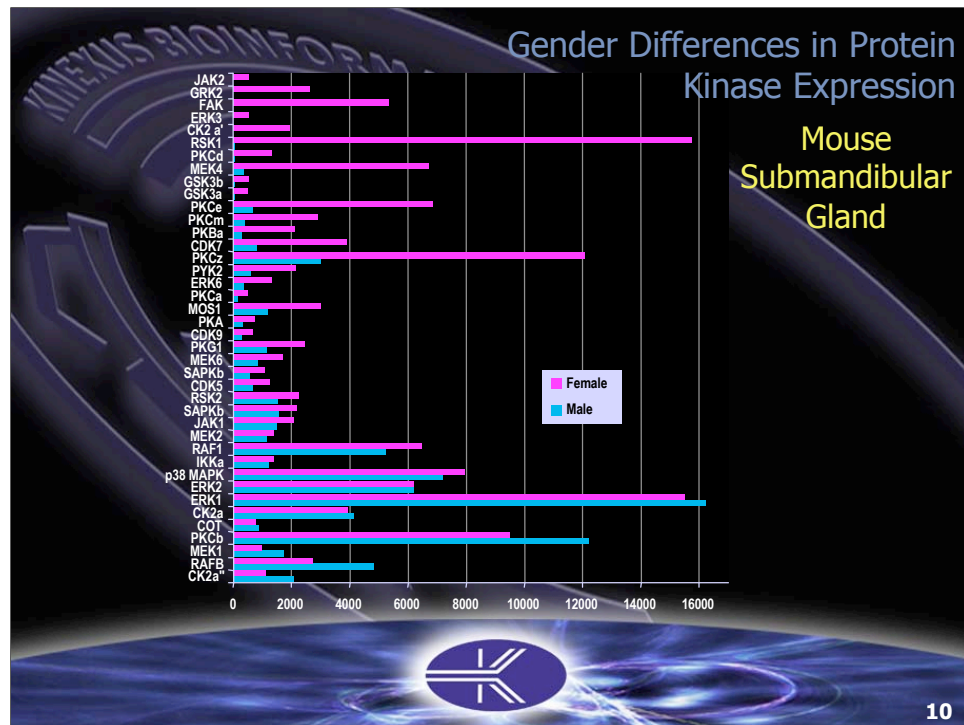


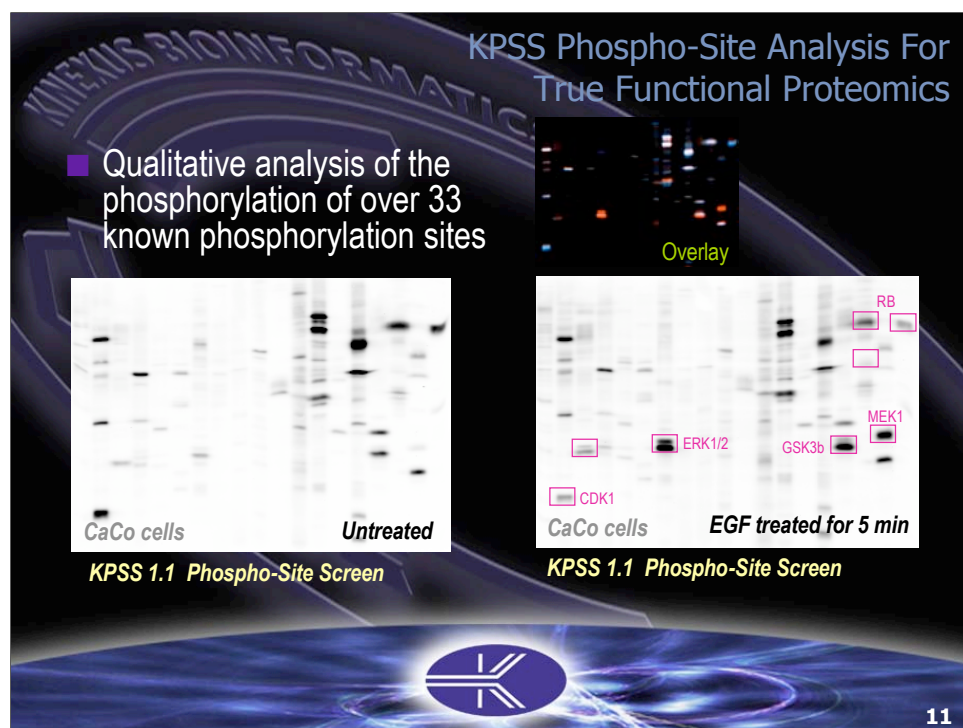
In the Science Magazine's Signal Transduction Knowledge Environment (STKE) study, Kinetools™ analysis(www.stke.org/cgi/content/full/sigtrans;2002/162/pe50), This and the following slide demonstrate the importance of tracking potential drug targets in diverse organs, since the expression patterns of protein kinases are not readily predictable. Furthermore, we have recently observed large differences in protein kinase expression patterns between male and female rats for the same somatic tissues. More than a third of the protein kinases showed expression changes of greater than 5-fold in the same types of organs. These unexpected differences between species may account in part for why drugs that perform well in pre-clinical animal trials fail in human clinical studies.



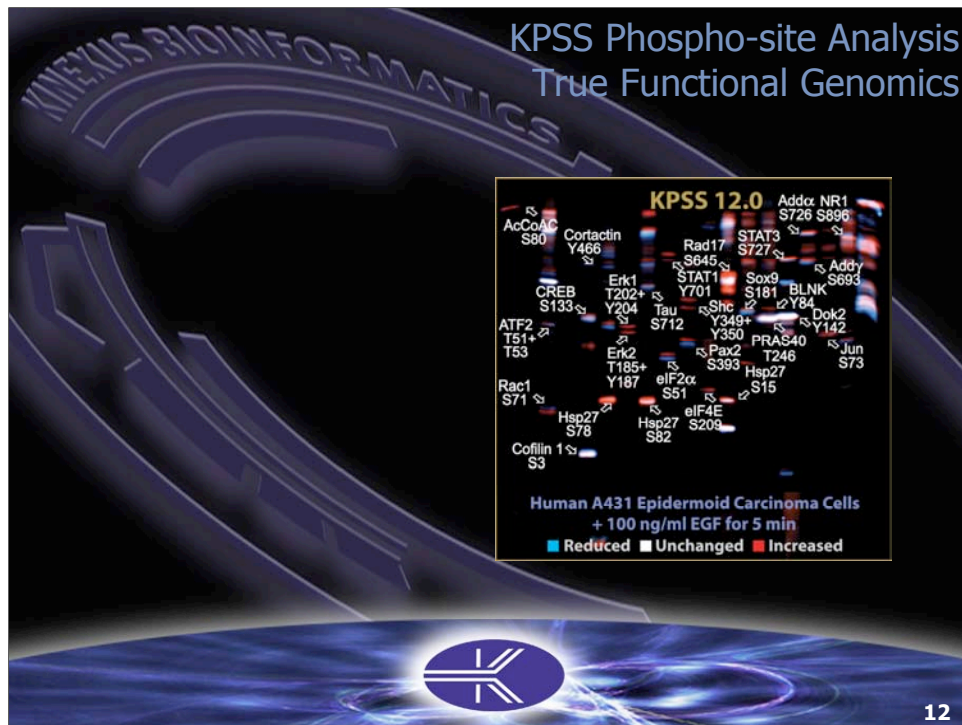


We have recently explored the effect of gender on patterns of protein kinase expression in somatic tissues from reproductive-competent male and female rats using the Kinetools KPKS 1.3 Protein Kinase Screen. The results shown in slide for mouse brain and the following slide for mouse submandibular gland demonstrate that more than half of the protein kinases detected in each tissue displayed greater than 2-fold differences in expression for age-matched mice. The implications for this are profound, as it reveals vast differences in the composition and architecture of protein kinase networks between males and females, and this could result in differential responses to drugs.

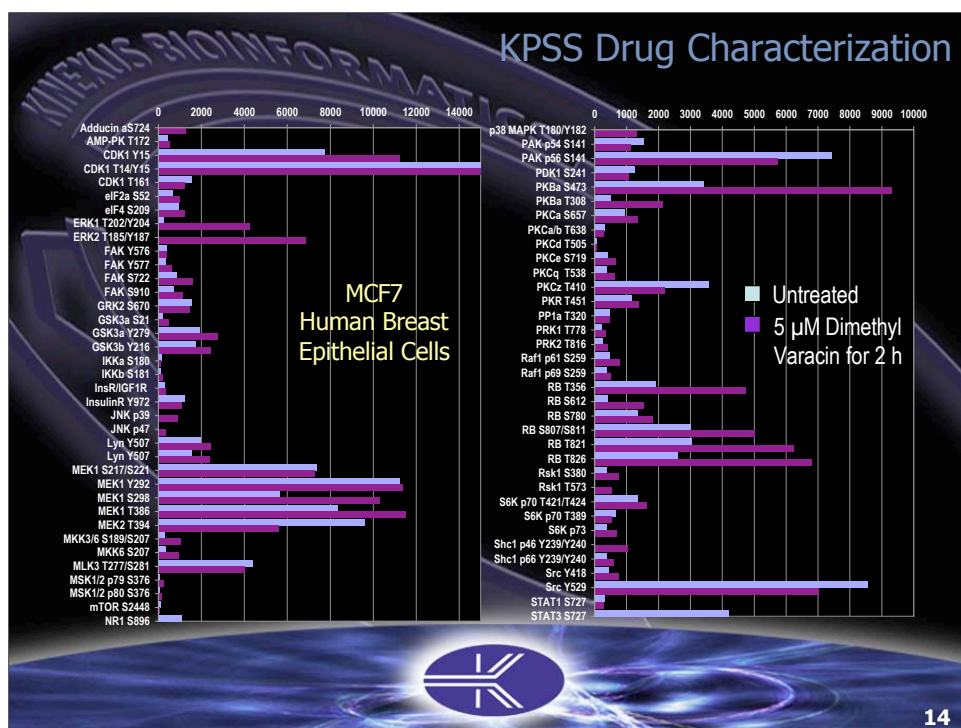




The Kinetworks™ KPSS 1.3 Phospho-Site Screen permits the tracking of 33 known phosphorylation sites with specific antibodies. We also provide 7 other phospho-site screening services for different phosphoproteins in the KPSS series. In this example, as expected, EGF treatment of the human colon carcinoma CaCo cell line leads to rapid phosphorylation of MEK1 and ERK1/2 at their activation sites. However, in the same experiment, the KPSS 1.3 screen revealed an unexpected reduction in the phosphorylation of the Thr-161 activation site of CDK1 and enhanced phosphorylation of Tyr-216 activation site of GSK3-beta. RB serine phosphorylation was also reduced by EGF treatment. Kinexus has the capability to track over 200 different known phosphorylation sites, but over 500 phosphoproteins can be detected with the KPSS 1.3 and 10.1 to 12.1 screens.

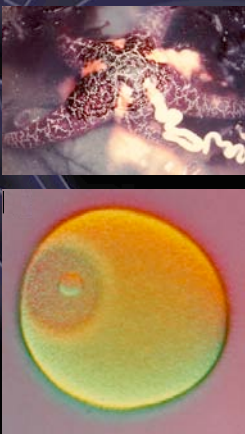


This slide shows with the KPSS 12.0 Phospho-Site screen enhanced epidermal growth factor phosphorylation of a wide range of proteins in the human cervical carcinoma cell line A431, in which the EGF-receptor is over-expressed. EGF-treated A431 cell blots shown in red are overlaid with blots from untreated A431 cells shown in blue. Approximately half of the changes detected novel proteins that could be easily identified. The availability of phospho-site antibodies for their enrichment and detection permits their purification for mass spectrometry identification.




This slide demonstrates the power of the Kineteworks™ KPSS Phospho-Site screens to uncover the mechanism of actions of drugs. In this case, dimethyl varacin, a cell cycle inhibitor, was found to markedly enhance the phosphorylation of the MAP kinases ERK1, ERK1, p38 and JNK, without increasing the phosphorylation of the MAP kinase kinases (MEK's) that phosphorylate these MAP kinases. These results support the hypothesis that the site of action of dimethyl varacin is inhibition of the dual specificity phosphatases that target MAP kinases. This is currently being followed up by our collaborators.

Drug Target Discovery - Proof of Concept of Strategy



- Detected major tyrosine dephosphorylated protein during seastar oocyte maturation with a phospho-site antibody
- Purified and identified the protein by sequencing as p38 MAP kinase
- Over 12 companies now involved with p38 inhibitors in clinical trials

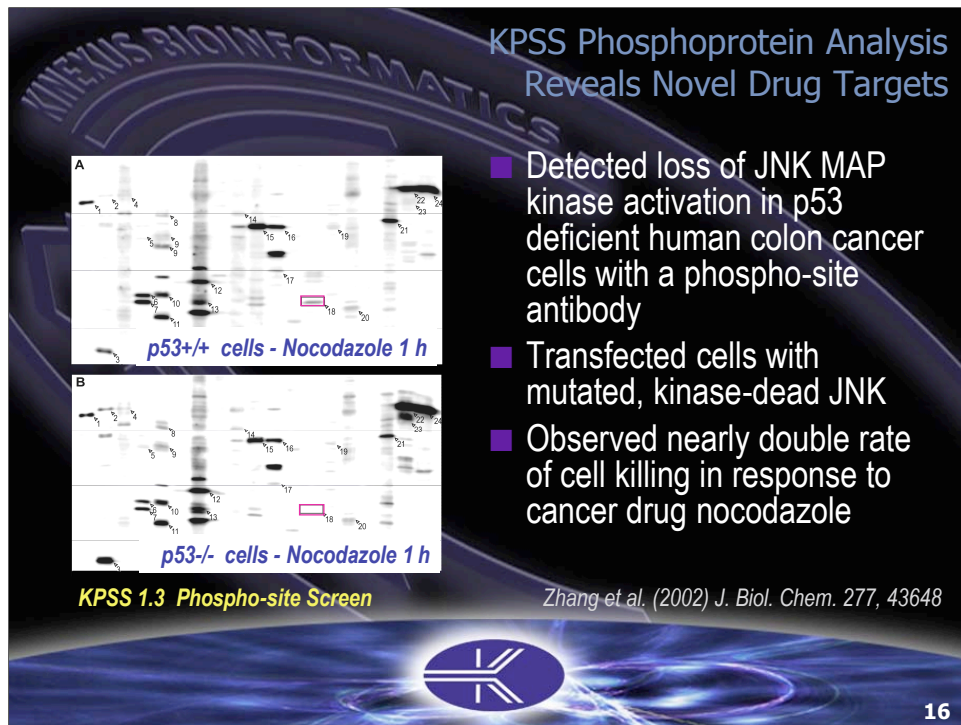
Morrison et al. (2000) J. Biol. Chem. 275, 34236



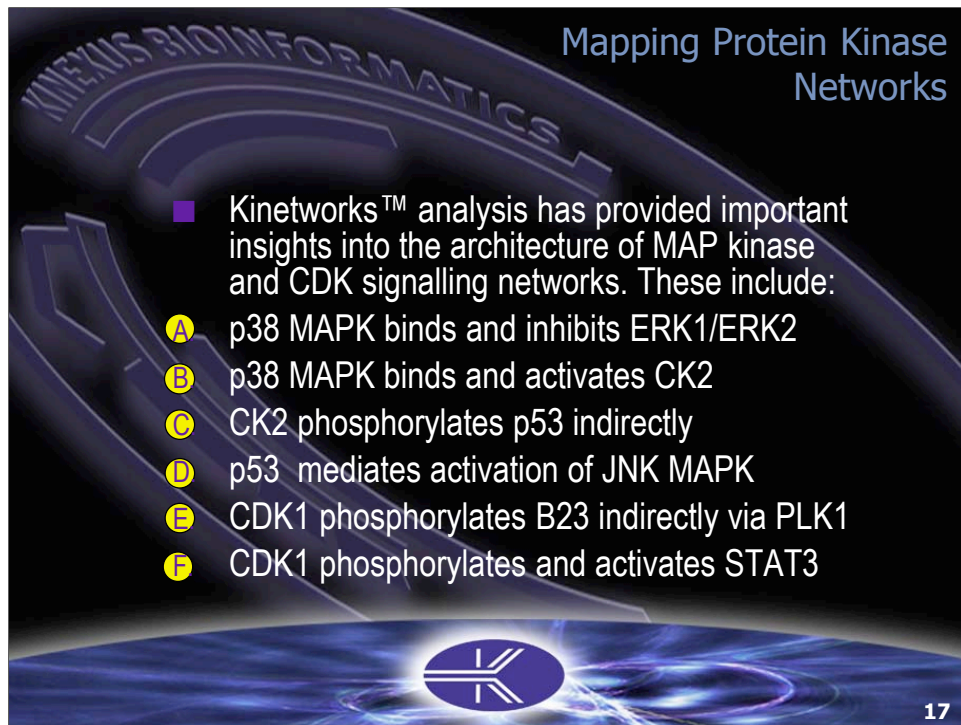
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There are several examples already for how the Kinexus strategy with our Kinetworks™ proteomics services can be used successfully to identify drug targets.

In one example, we detected an unknown protein that underwent tyrosine dephosphorylation when oocytes from starfish underwent conversion into fertilizable eggs. We discovered that this protein could also be detected with an antibody in our panel that corresponded to a protein kinase. Encouraged by this observation, we used this antibody to purify the protein sufficiently so that we could successfully identify it by mass spectrometry. It turned out to be a very important protein kinase that amongst other things controls inflammation. There are already at least 12 companies that now have inhibitors of this protein kinase in clinical studies. Although this kinase was identified previously as a drug target, our findings validate the use of the Kinetworks™ approach for protein kinase drug target discovery.



We used our Kinetools™ KPSS screen to detect and identify drug targets. Using this screen, we analyzed the ability of a cancer drug called nocodazole to alter protein phosphorylation patterns in colon cancer cells that are either sensitive (i.e. p53+) or resistant (i.e. p53-) to killing by nocodazole. This drug dissociates microtubules found in the spindle apparatus and prevents mitosis in p53+ cells. We found that there was a specific reduction of phosphorylation and activation of a protein kinase called JNK that correlated with loss of p53 function. When we abolished JNK function in the nocodazole-sensitive, p53+ cells, we actually observed enhanced rate of cell killing. Prior to our studies, JNK was generally thought only to promote cell death, but to the contrary, our findings point to JNK as an excellent drug target for treatment of some forms of solid tumour cancers.

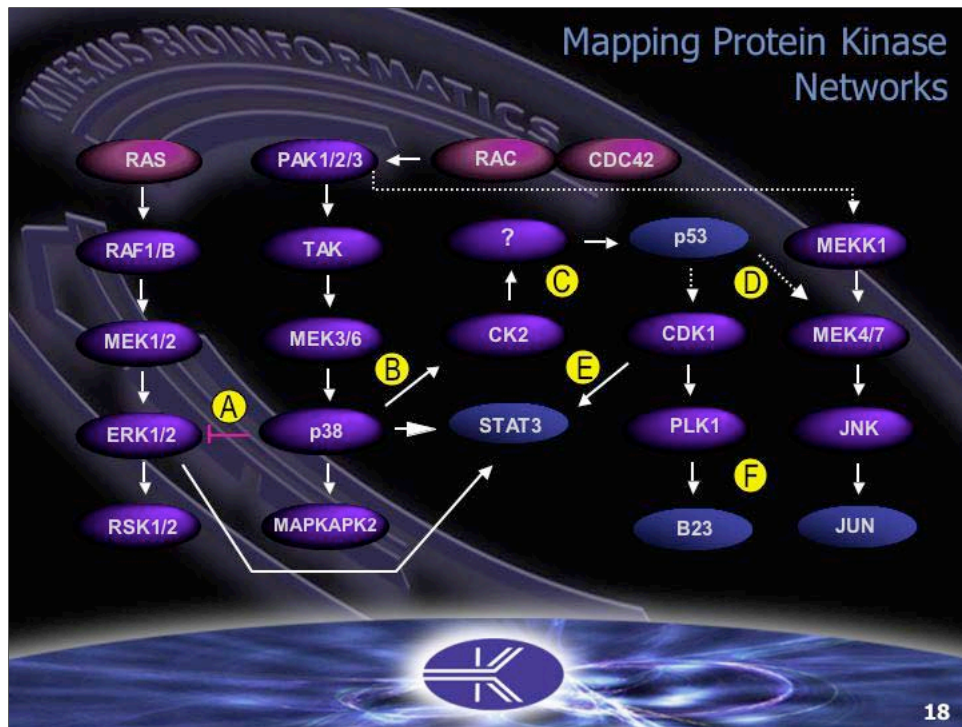


Mapping Protein Kinase Networks

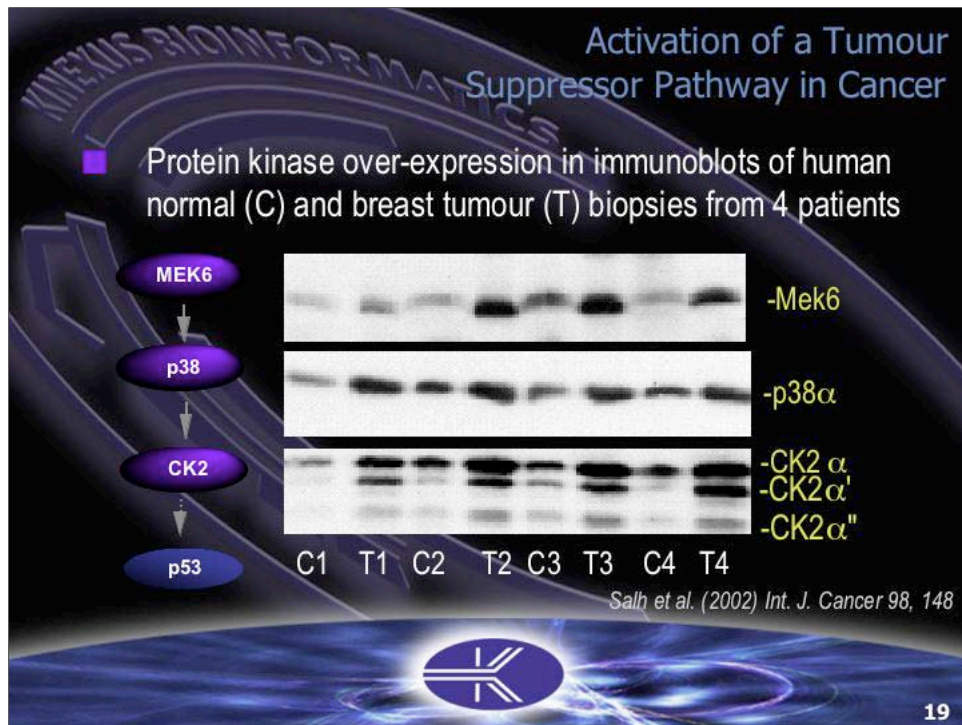
- Kinetworks™ analysis has provided important insights into the architecture of MAP kinase and CDK signalling networks. These include:
 - A p38 MAPK binds and inhibits ERK1/ERK2
 - B p38 MAPK binds and activates CK2
 - C CK2 phosphorylates p53 indirectly
 - D p53 mediates activation of JNK MAPK
 - E CDK1 phosphorylates B23 indirectly via PLK1
 - F CDK1 phosphorylates and activates STAT3

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Over the last five years, we have applied the Kinetworks™ approach to uncover many novel connections between different signalling pathways for MAP kinases and cyclin-dependent kinases. The next few slides provide several examples of where several important insights have been generated and described in scientific publications (reprints are available on this CD) or patent applications.

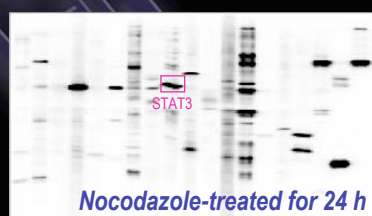


This slide summarizes the important connections that we have recently established between protein kinases involved in mitotic control.



Kinetworks™ custom analysis has revealed the overproduction of the protein kinases CK2, p38-alpha MAP kinase and Mek6 in solid human tumours from the breast, lung, liver and colon. While some companies have considered one or more of these protein kinases as drug targets, our findings tend to invalidate these as suitable candidates. Our data shows that these kinases are integrated into a pathway that becomes up regulated in cancer cells to compensate for a loss of p53 function. Inhibition of this tumour suppressor pathway by drugs would probably serve to worsen the cancer prognosis.

Drug Target Discovery



- Detected increased phosphorylation of STAT3 at Ser-727 in response to nocodazole treatment of HeLa cells

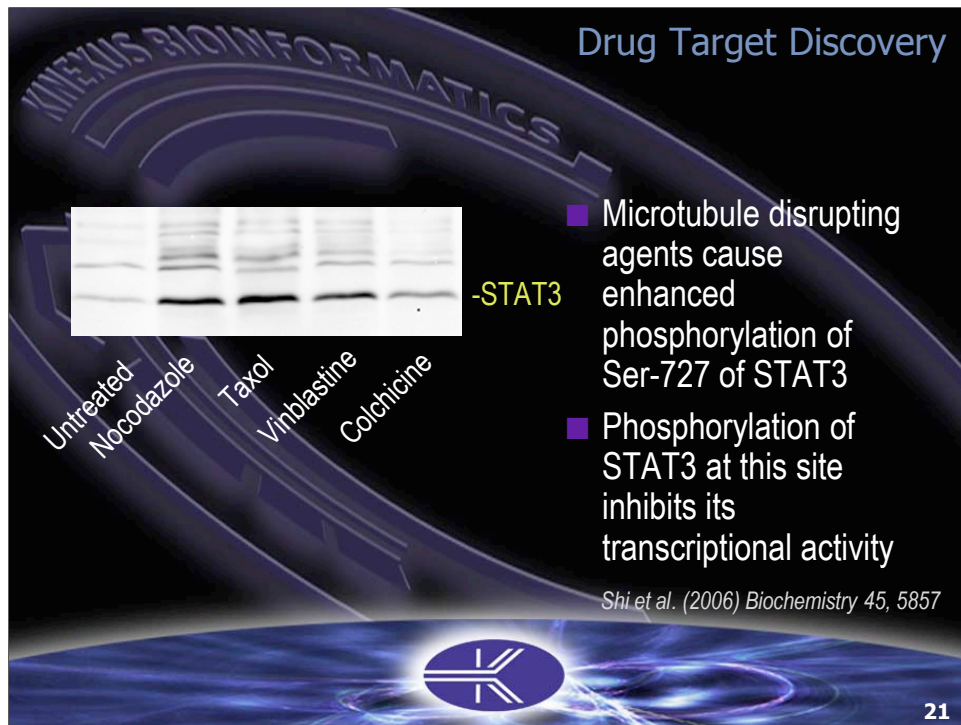
KPSS 1.3 Phospho-site Screen

Shi et al. (2006) *Biochemistry* 45, 5857



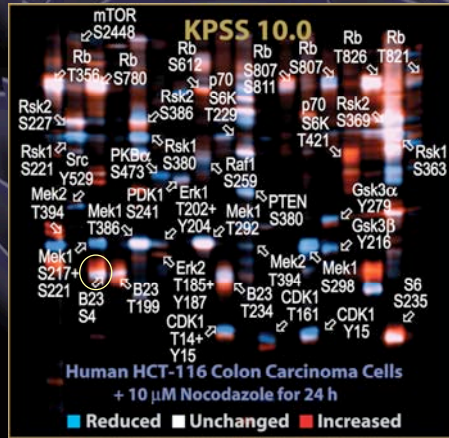
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In another example of the usefulness of the Kinetix™ analyses to uncover novel phosphorylation events, we used our KPSS 1.3 Phosphoprotein Screen to detect proteins that underwent enhanced phosphorylation in response to treatment of an established human cervical cancer cell line to a cancer drug. One of the proteins that showed increased phosphorylation in response to nocodazole treatment for 24 hours in HeLa cells was STAT3 at Ser-727.



The effect of nocodazole and other disruptors of microtubule dynamics to enhance STAT3 Ser-727 phosphorylation in HeLa cells could be reproduced in Western blots of cell lysates probed with the STAT3 Ser-727 phospho-site antibody. We have recently demonstrated that this appears to be partly mediated through direct phosphorylation by CDK1. Previous to these studies, there have been no reports in the literature that STAT proteins are phosphorylated on serine during mitosis.

Drug Target Discovery

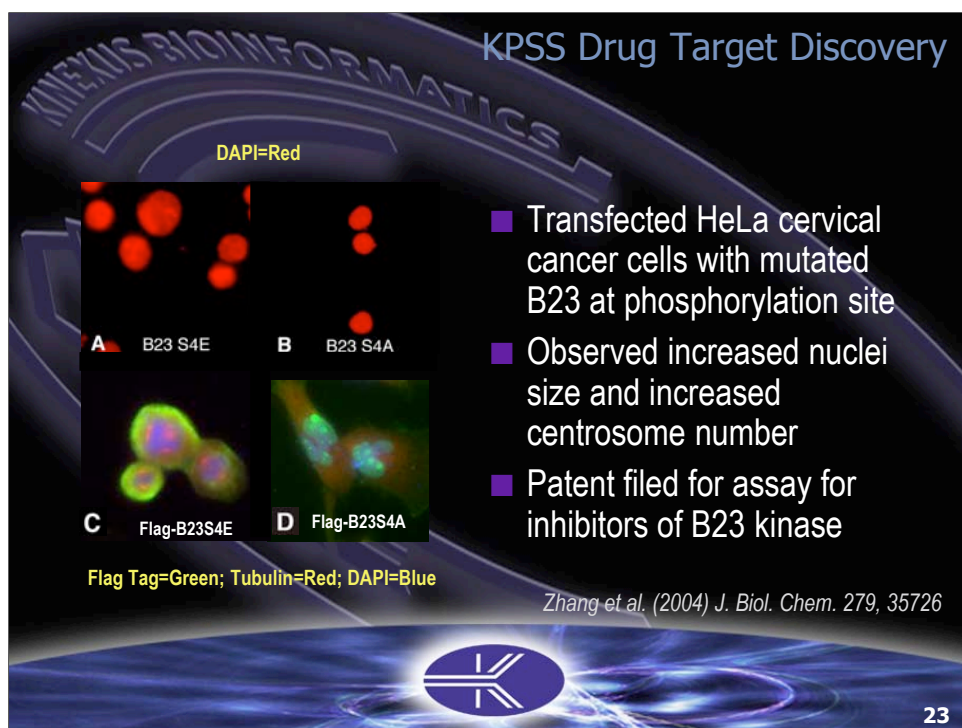


- Detected increased phosphorylation of unknown protein in human colon carcinoma HCT-116 tumour cells in response to a cancer drug with a phospho-site antibody
- Purified and identified protein by mass spectrometry as B23 (also known as nucleophosmin)

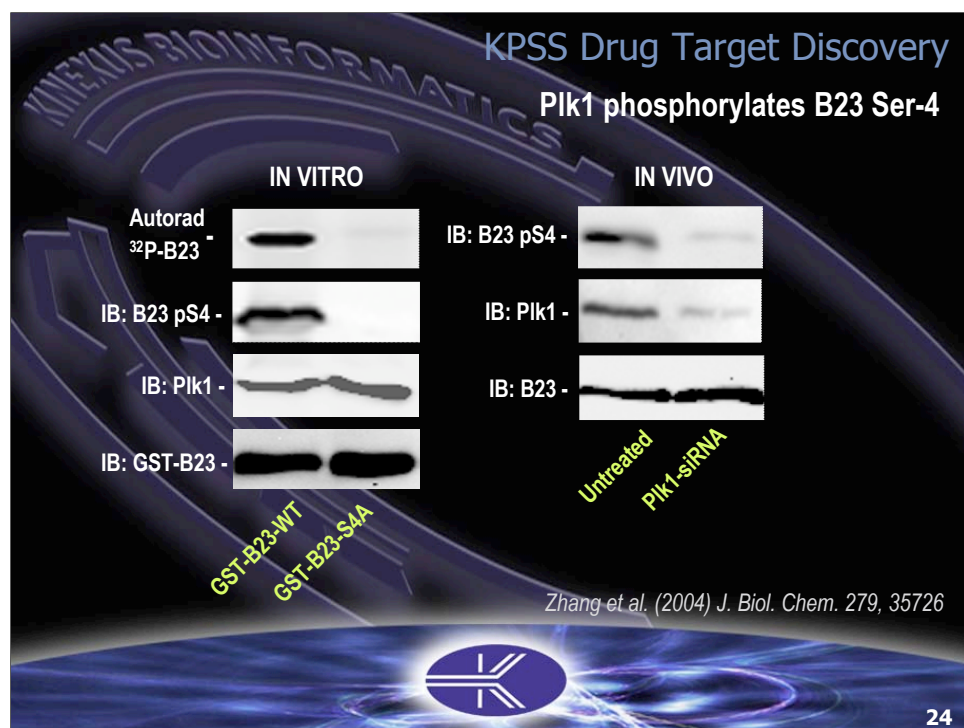
Zhang et al. (2004) J. Biol. Chem. 279, 35726

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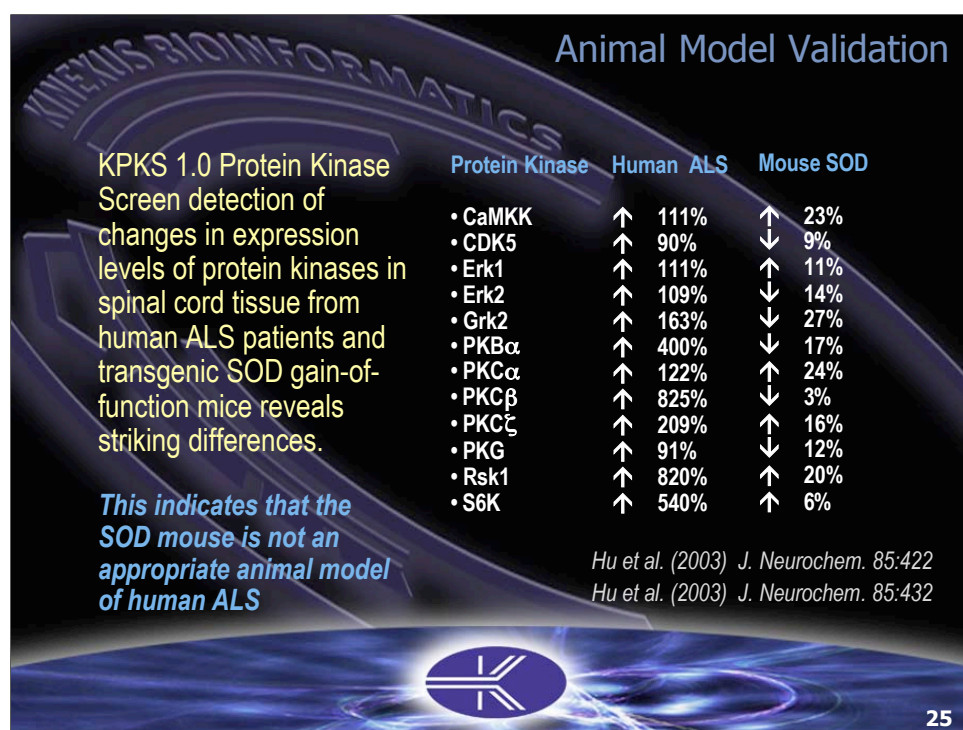
With nocodazole arrest of many cell lines, as shown above with HCT-116 cells, we also detected the enhanced phosphorylation of a previously unidentified 40 kDa phosphoprotein (circled in yellow) using our KPSS phospho-site screens. This protein cross-reacted with a phospho-site antibody developed to recognize Mek1 at S217 and S221, and it was identified as B23 using mass spectrometry. This protein was previously demonstrated to be highly produced in the nuclei of proliferating cells.



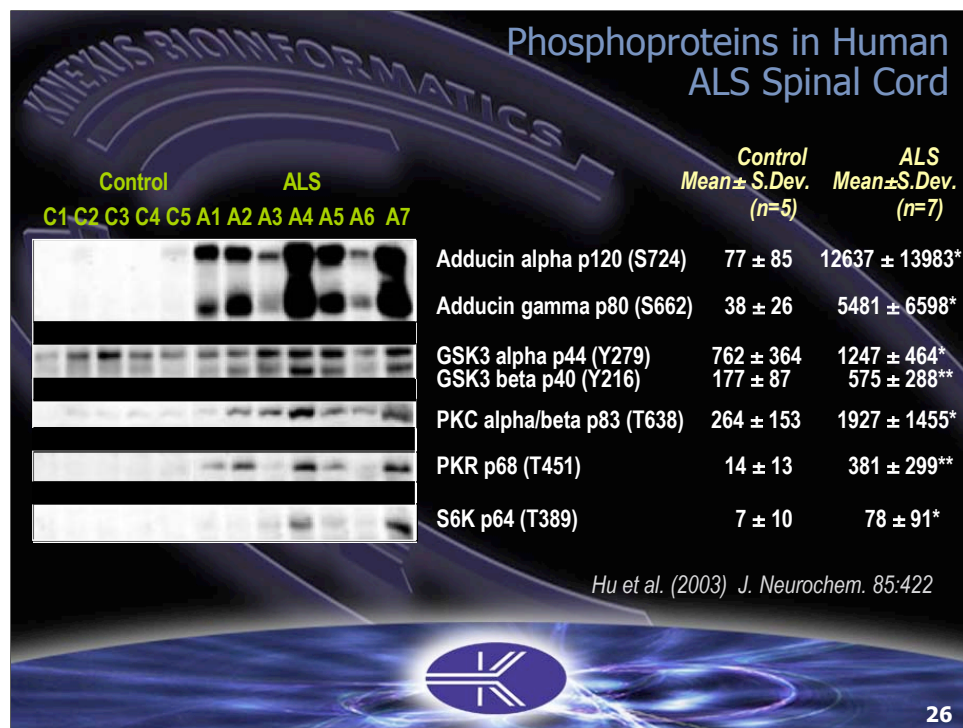
Since we knew the epitope of the phospho-site antibody that cross-reacted with the B23 protein, we were immediately able to assign the amino acid residue that was phosphorylated as Ser-4. When HeLa cells were transfected with a mutant B23 that could not be phosphorylated [Ser-4 to Ala-4], there was only one or no centrosomes detectable per cell. By contrast, transfection with a mutant B23 to mimic constitutive phosphorylation at this site [Ser-4 to Glu-4] resulted in up to six centrosomes per cell.



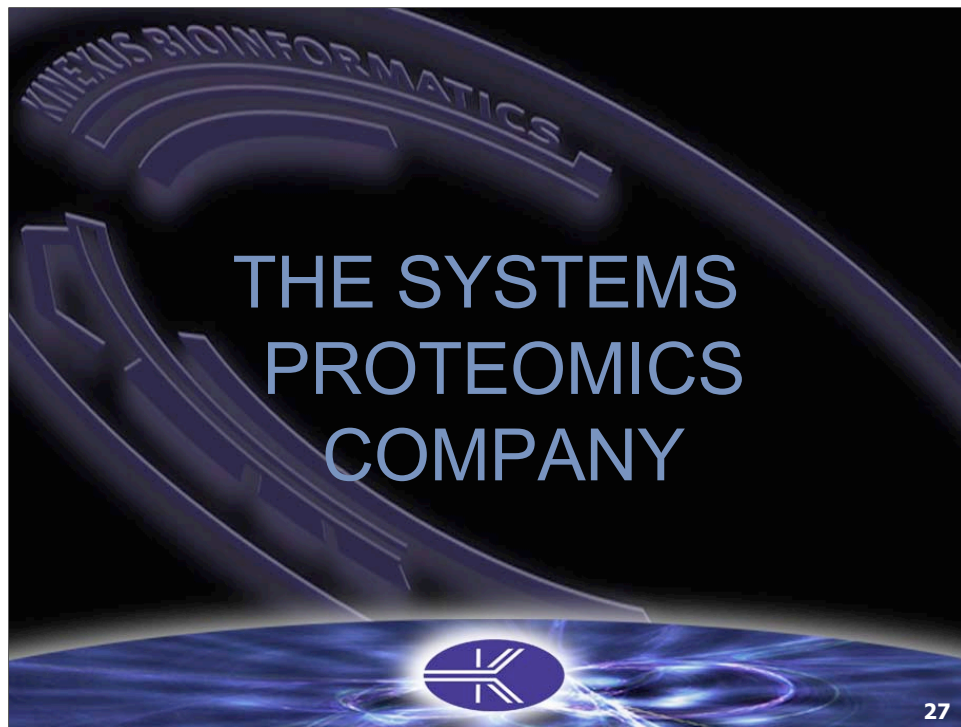
The Ser-4 site in B23 fitted the consensus phosphorylation site recognition sequence for the polo-like kinase (Plk). We were able to demonstrate that Plk1 was phosphorylated and activated in response to nocodazole in HeLa cells, and that this was mediated by cyclin-dependent kinase 1. In summary, our study identified a novel non-radioactive assay for Plk1 and validated it as an appropriate target for drug discovery.



More than four out of five drug leads that look promising in animal studies fail in clinical trials. This is because the animal models of the disease are not reflective of the human situation. We recently completed a Kinetools™ study in which we discovered striking changes in the levels of protein kinases and the phosphorylation states of targets for these kinases in spinal cord samples from patients who have died from amyotrophic lateral sclerosis (ALS), a devastating neurodegenerative disease. When we examined the leading transgenic mouse model for this disease, which is used extensively in preclinical studies of lead drugs to treat ALS, we showed profound differences in the molecular changes in cell signalling in the established mouse model. Our findings question the appropriateness of this animal model, and our Kinetools™ analysis could be a useful tool for the identification of more relevant animal models for this and other diseases.



When the Kinetix™ KPSS 1.3 Phospho-Site screen was used to track phosphoprotein changes in spinal cord sections of patients that died from ALS, there were several changes in ALS (A) samples as compared to spinal cord samples from controls (C). In particular, there was a more than 100-fold increase in the phosphorylation of adducin at the site targeted by protein kinase C. This was one of the protein kinases that were elevated about 9-fold in expression in ALS. Our findings point to protein kinase C as a potential drug target for treatment of ALS.



We hope that these slides offer a sense of the power of our proteomics services. We feel that our services can position our clients at the cusp of a profound paradigm shift in the way that research is conducted today and ultimately we hope to contribute to the realization of the goal of the delivery of personalized medicine. We believe that by eavesdropping on the molecular communications systems that operate in all of the living cells of the human body, we can learn more about what has gone awry in these cells during disease and assist in the healing process in a more rational way based on deeper understanding. This will revolutionize medicine and improve human well being in ways that have been unprecedented and not previously feasible.