Mass Spectrometry Based Proteomics
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Mod 04 Lecture Number 19

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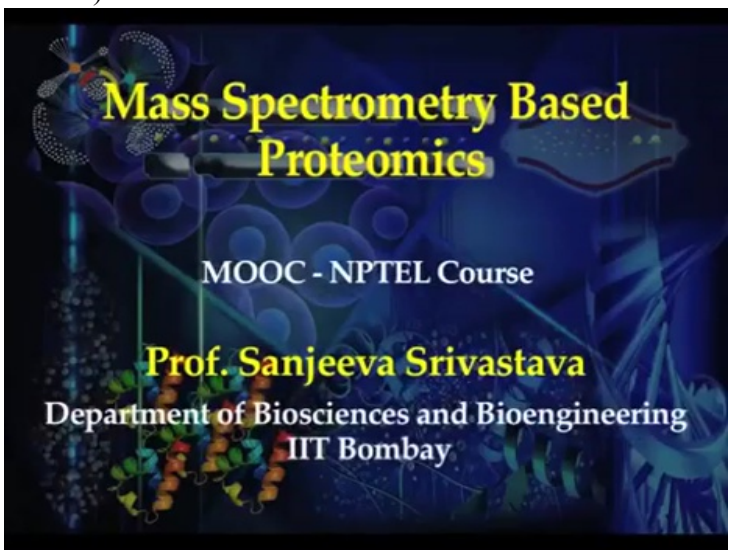

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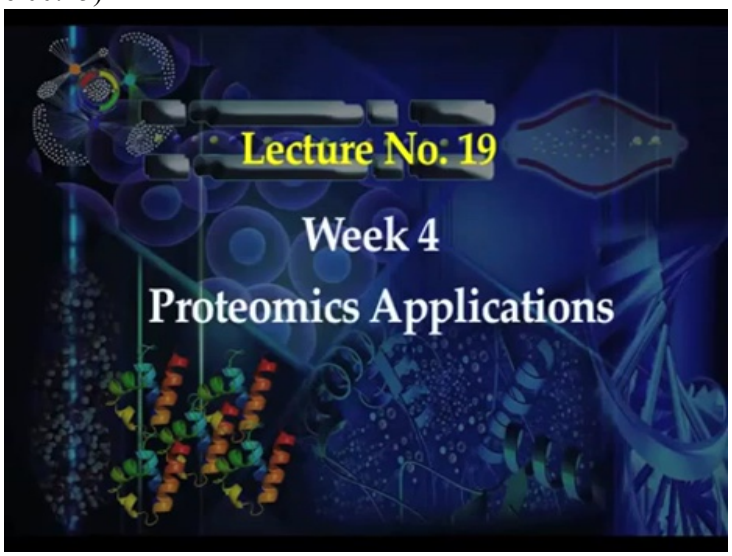

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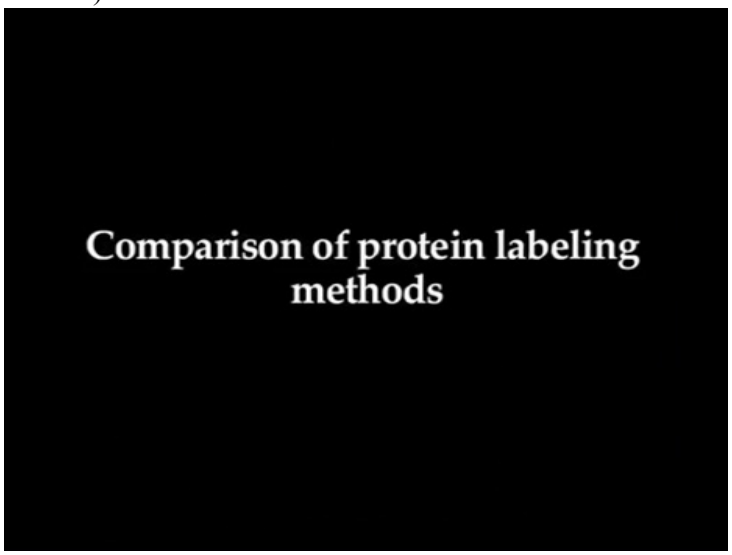

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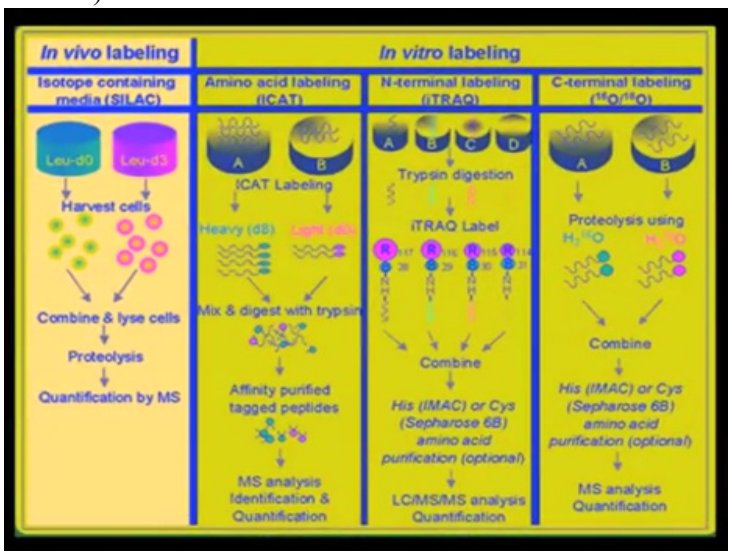

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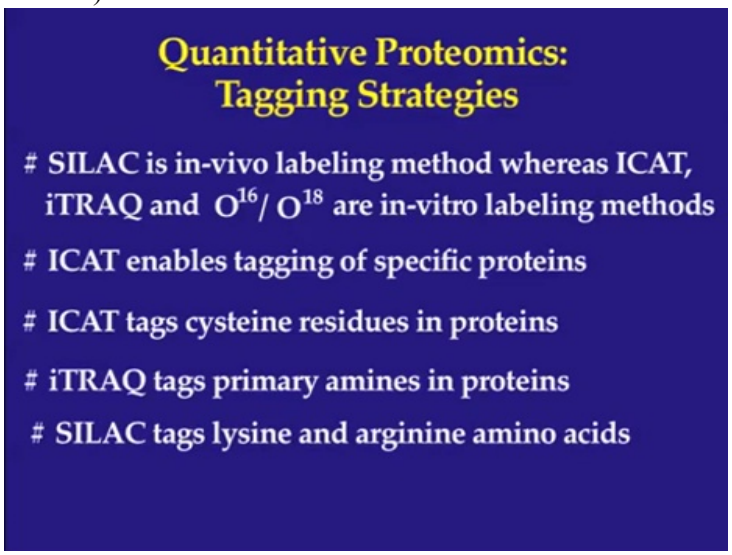

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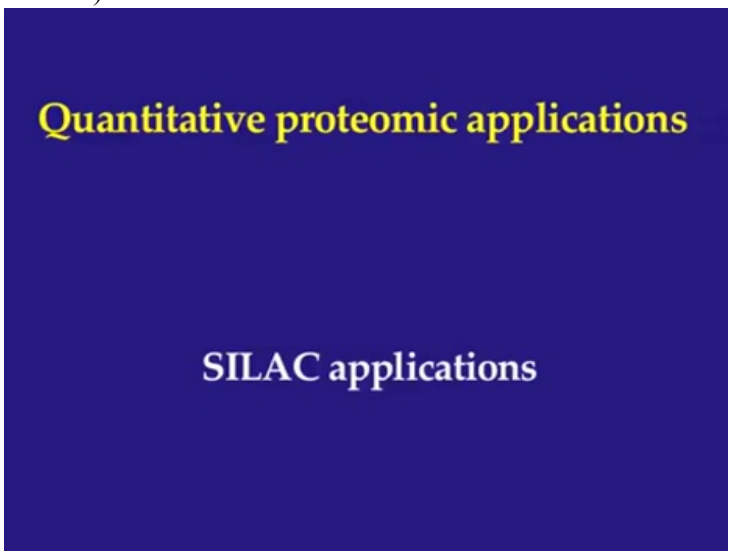

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Let us now discuss few applications of SILAC briefly.

The SILAC method is very promising for any cell line. So this method can be applied for

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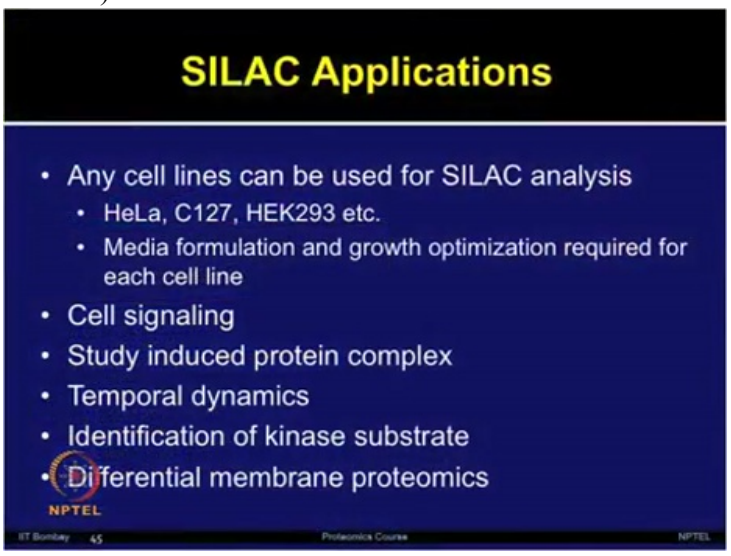

…any cell line, whether it is HeLa cell, C127, HEK293 or different types of cell lines people have shown.

However, the media formulation and the growth optimization is required individually for each cell line.

SILAC applications have been demonstrated in different applications such as cell signaling, studying the induced protein complexes, studying temporal dynamics, identification of kinase substrates, studying differential membrane proteomics.

So there are various applications we will have a look on the some applications now.

Ong et al in 2002 published a paper in cell proteomics which was the first SILAC application demonstrated,

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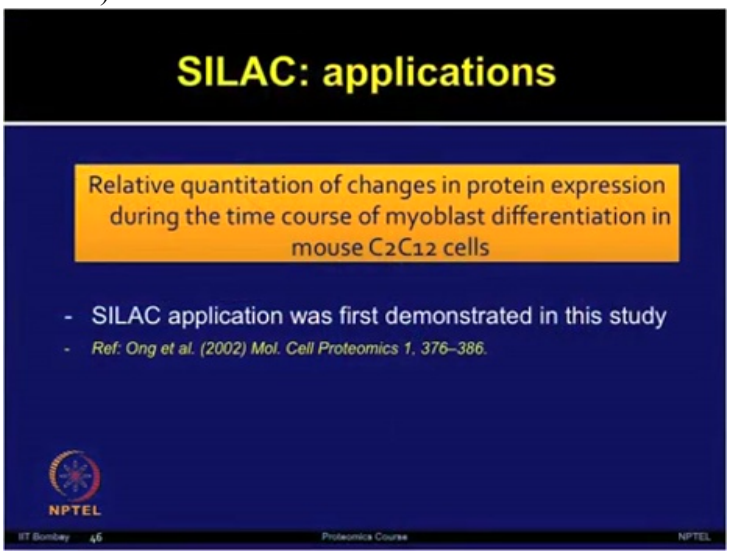

… where they used the relative quantification of changes in protein expression during the time course of myoblast differentiation in mouse cells

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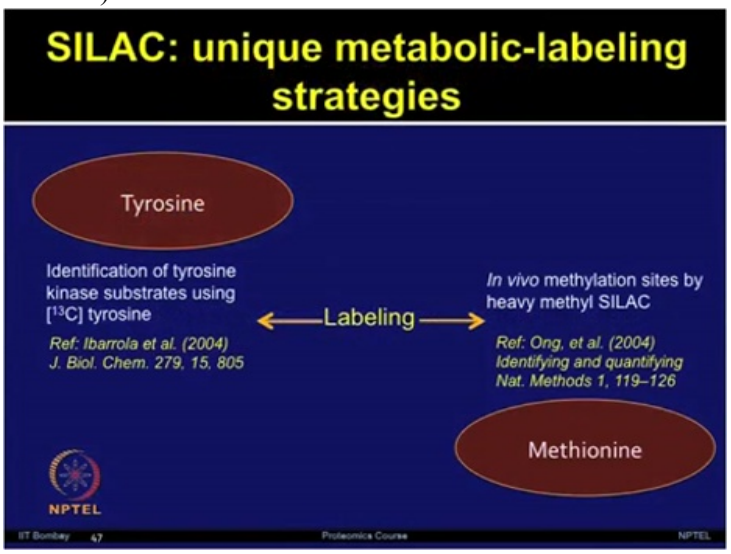

Researchers have reported various unique metabolic-labeling strategies.

For example, by using tyrosine; identification of tyrosine kinase substrates using 13C tyrosine Labeling is also performed by using methionine, the in vivo methylation sites by heavy methyl SILAC.

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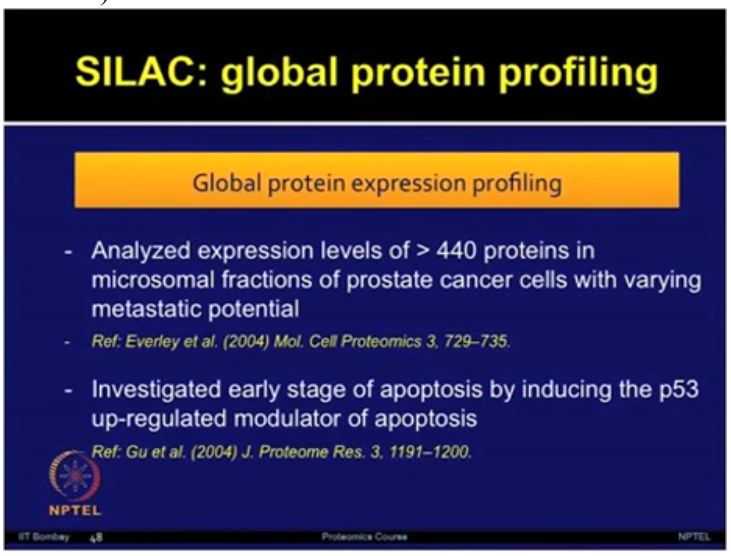

There are numerous studies based on the global protein expression profiling using SILAC method. I am just highlighting some of the very earlier studies which set up the path for performing these protein expression profiling.

So the study by Everley et al. in 2004 analyzed the expression levels of more than 440 proteins in the microsomal fractions of prostate cancer cells with varying metastatic potential.

Another study by Gu et al. investigated the early stage of apoptosis by inducing the p53 up-regulated modulator of apoptosis.

SILAC has also been used for functional assays to study the protein-protein interactions.
Study by Blagoev et al. used

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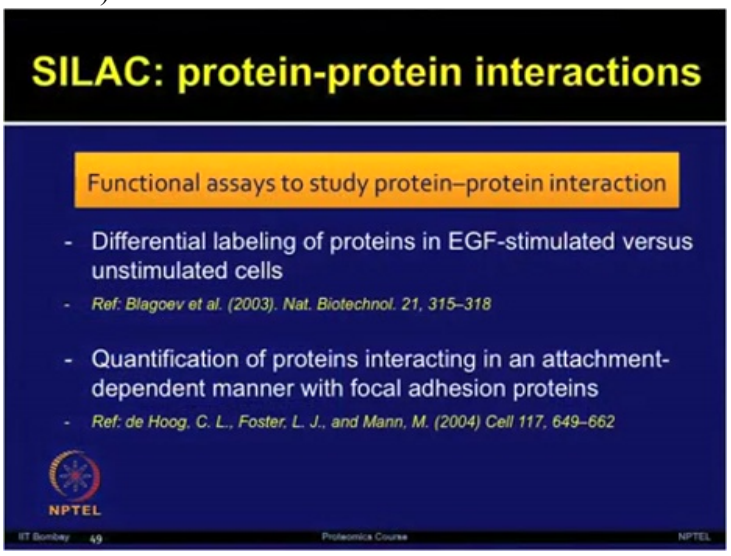

…the differential labeling of proteins in EGF-stimulated versus un-stimulated cells

Study by de Hoog et al. did quantification of proteins interacting in an attachment-dependent manner with focal adhesion proteins.

These are just few examples of studying the functional assays and performing protein interactions using SILAC.

The identification of proteins which are enriched in specific cellular structures; a study by Foster et al …

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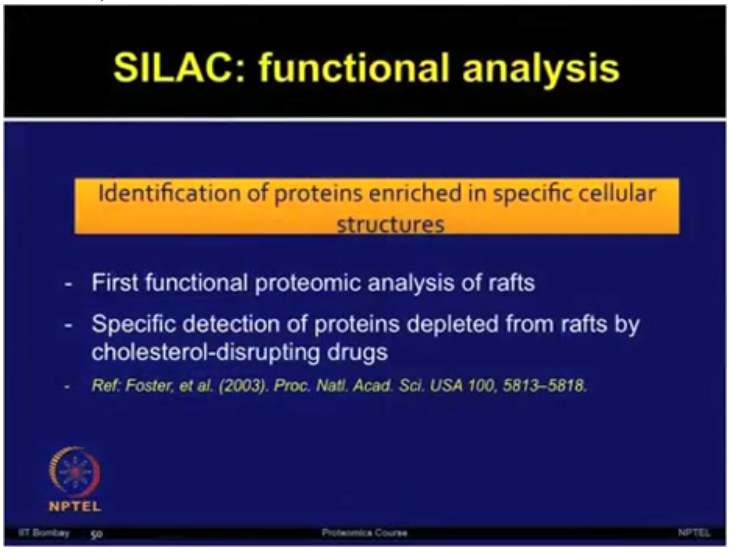

…used the first functional proteomic analysis of rafts and they showed that the specific detection of proteins depleted from the rafts by cholesterol-disrupting drugs.

SILAC has been widely used for multiplex analysis to compare the cellular states.

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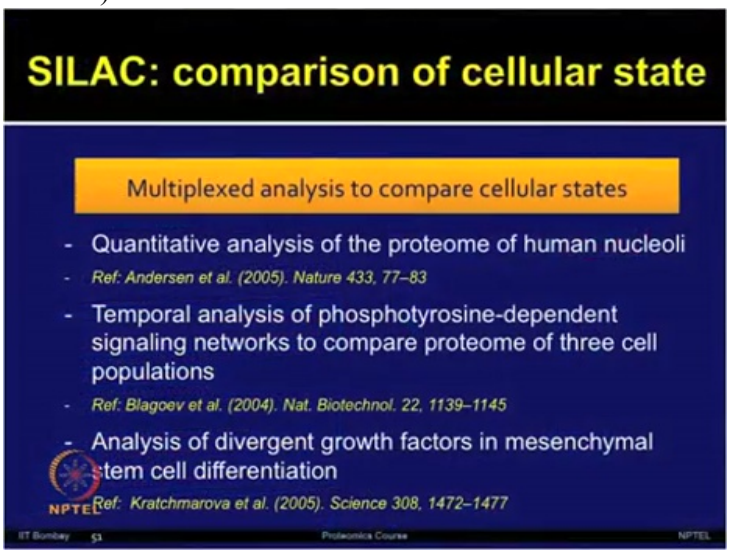

Andersen et al. showed the quantitative analysis of the proteome of human nucleoli.

Blagoev et al. performed a temporal analysis of phosphotyrosine-dependent signaling networks to compare the proteome of three cell populations.

Kratchmarova et al. analyzed the divergent growth factors in mesenchymal stem cell differentiation. These are just few examples of multiplex analysis.

Now if you look into literature, there are many studies which have used SILAC method for comparison of cellular states.

SILAC method has also been used to study the protein turnover.

Study by Pratt et al. used the rate of breakdown of individual proteins by analysis of mass shifts

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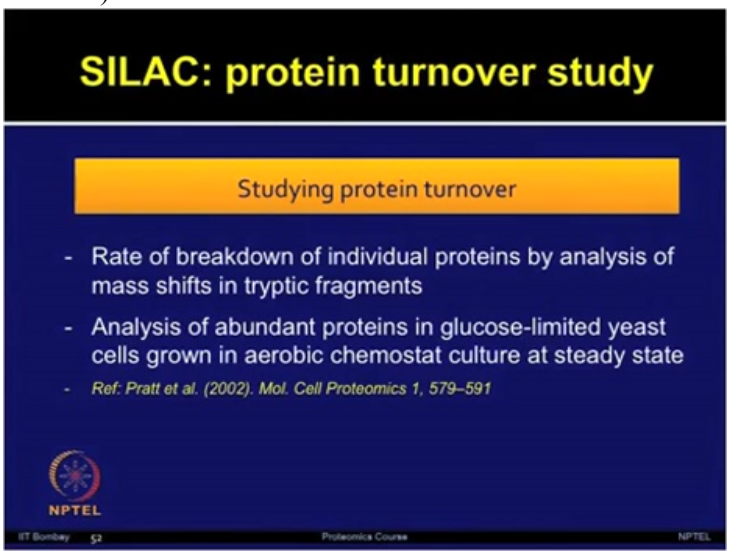

…in tryptic peptide fragments

The analysis of abundant protein in glucose-limited yeast cells which were grown in aerobic chemostat cultures at steady state was performed by using SILAC method.

SILAC has been used for identification and quantification of protein post-translational modifications.

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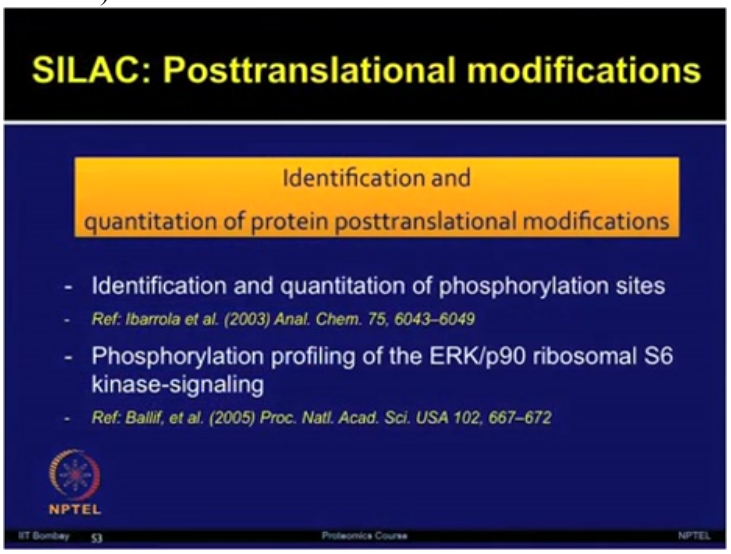

Study by Ibarrola et al. identified and quantitated phosphorylation sites.

Another study by Ballif et al. also identified and quantitated the phosphorylation sites. There are many studies which have used SILAC method for studying post-translational modifications.

Interestingly, now SILAC method has been used in different organisms; in bacteria…

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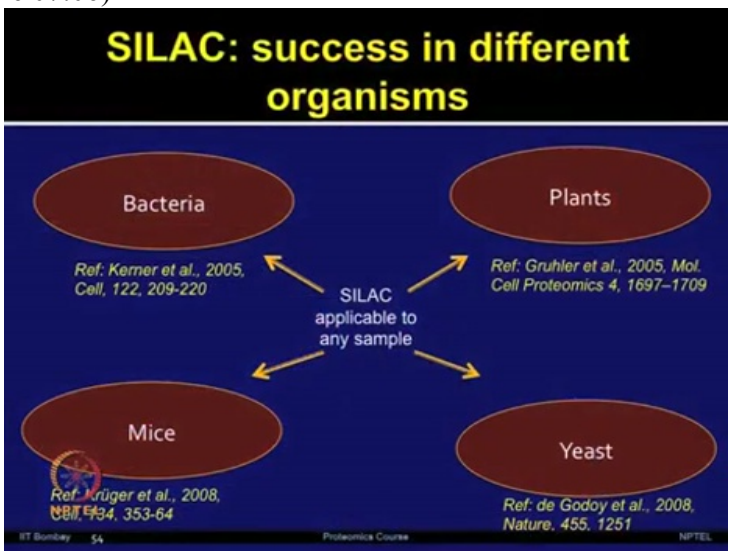

…in yeast, these were the more commonly used SILAC methods due to the growth in the cell culture.

But there are some studies on Arabidopsis in the plants, as well as in the mice which has shown that SILAC can be applied to the wide variety of organisms.

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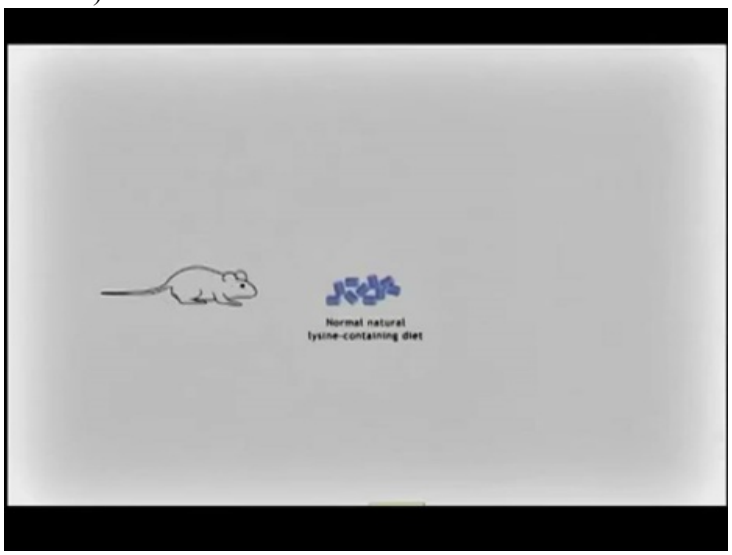

In an experiment conducted by Kruger and colleagues

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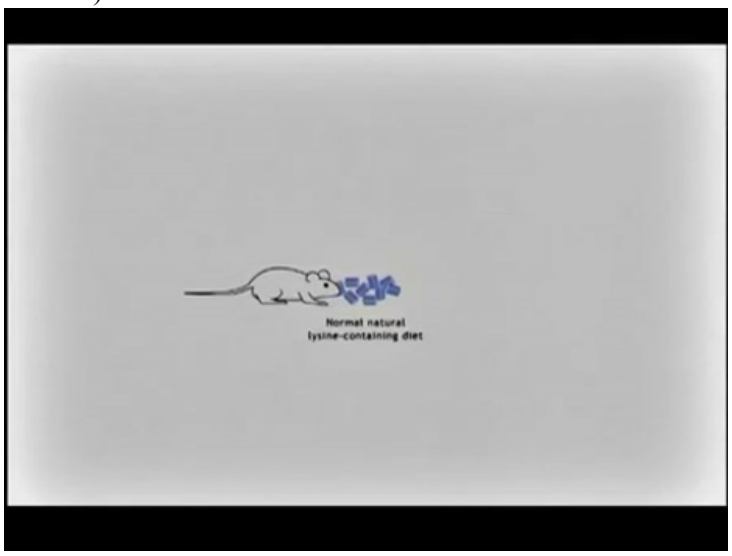

, a set of mice were fed

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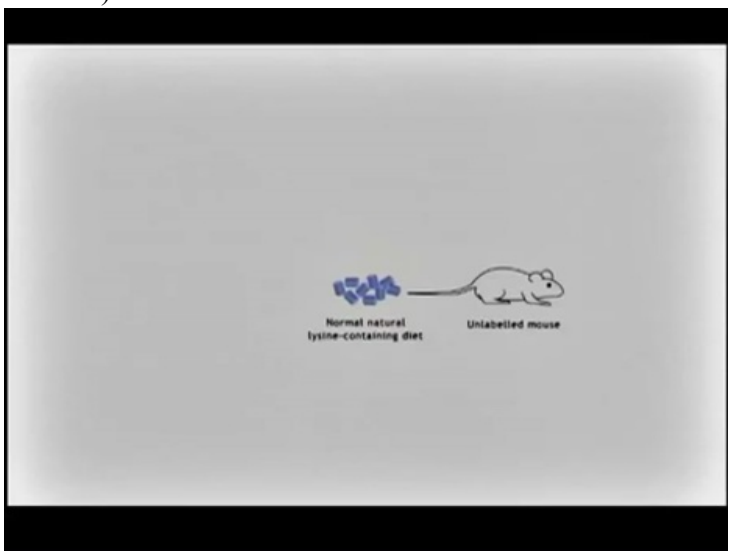

with diet containing natural lysine Another set of mice were fed

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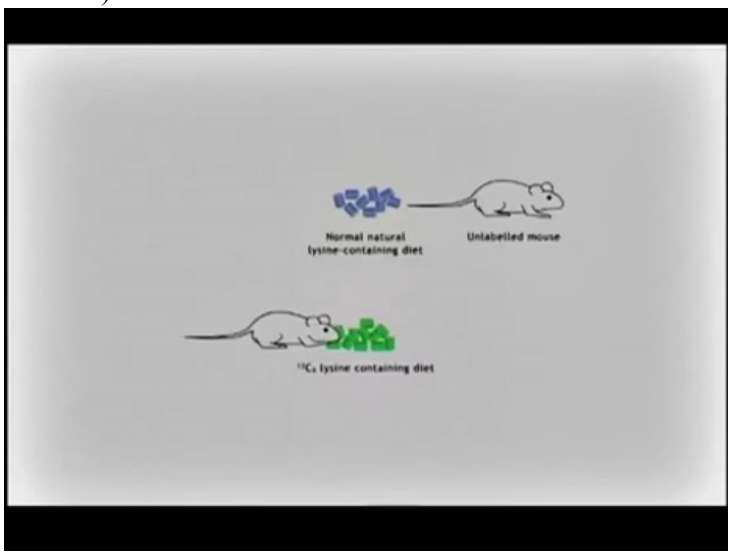

…with diet containing C13 labeled lysine.

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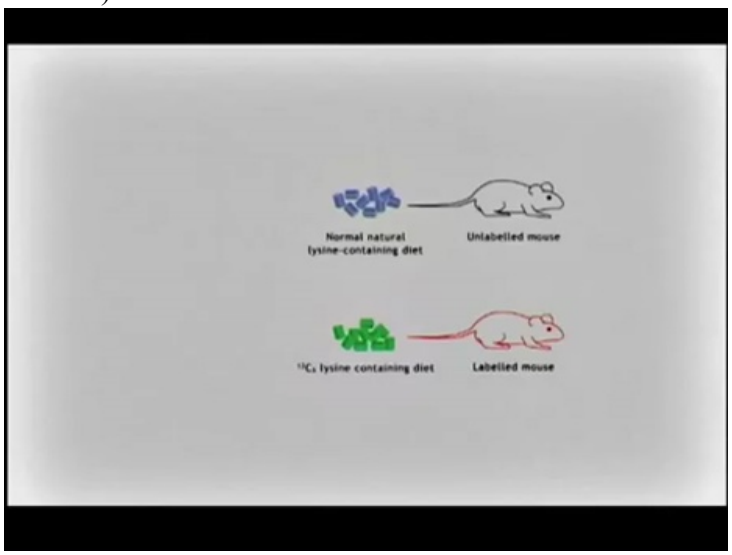

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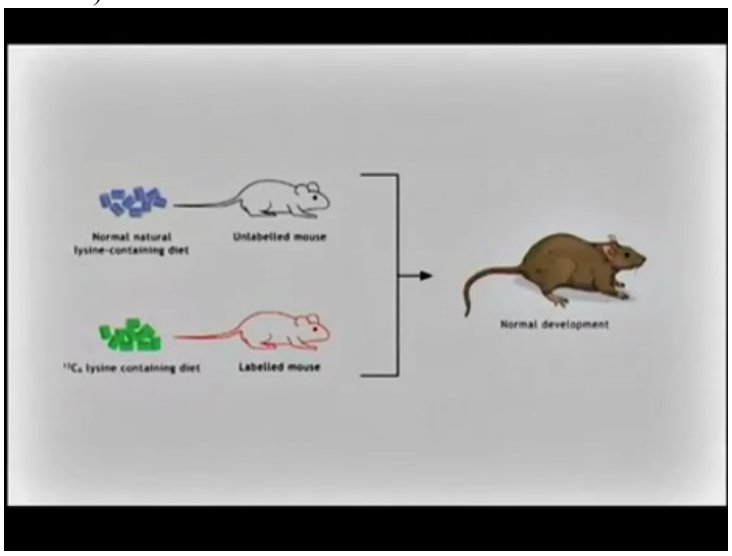

The researchers tracked the incorporation of Lysine 6 into the mouse proteome over 4 weeks by providing the C13 containing lysine dye.

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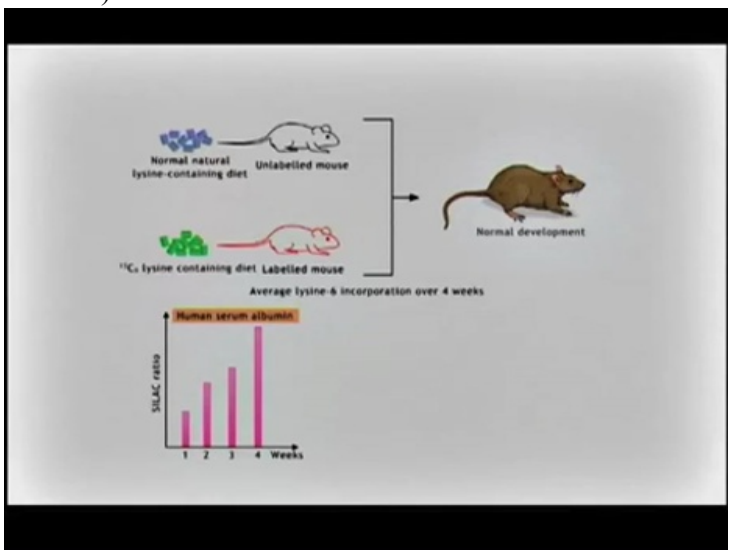

Their developmental growth and behavior were observed in addition to sampling various blood proteins. The labeled mice were found to develop normally

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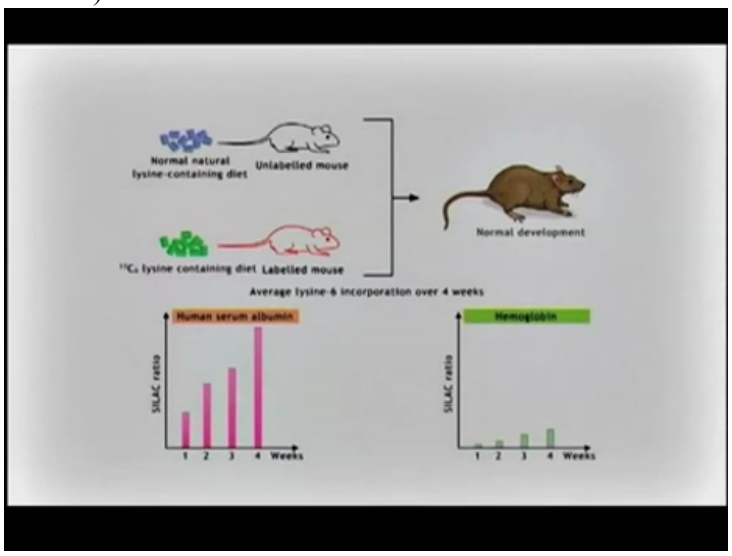

… average Lysine 6 incorporation over 4 weeks in human serum albumin and hemoglobin is depicted in the graph

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de Godoy and colleagues determined the fold change

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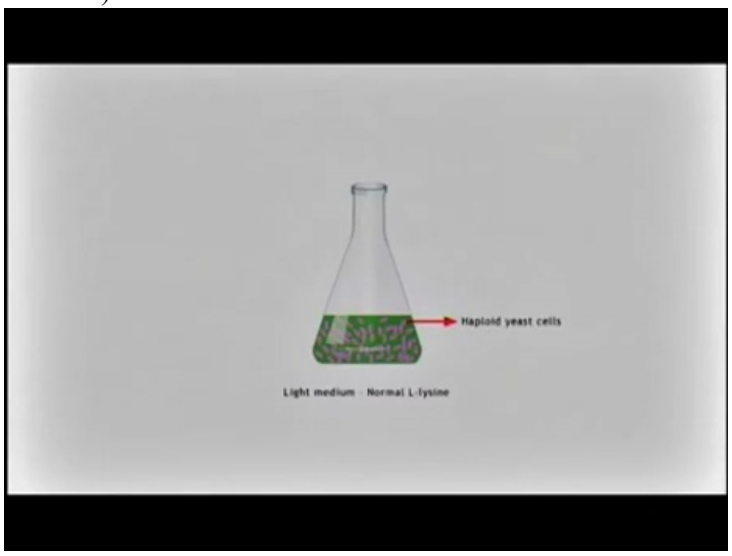

of peptide pairs between haploid and diploid yeast cells using SILAC. Labeled lysine residues were used to grow the diploid yeast cells

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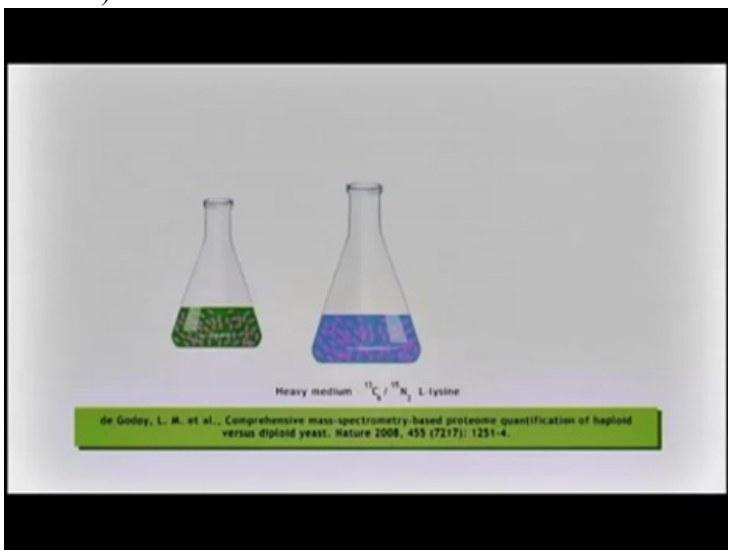

…while haploid cells were grown in normal lysine medium

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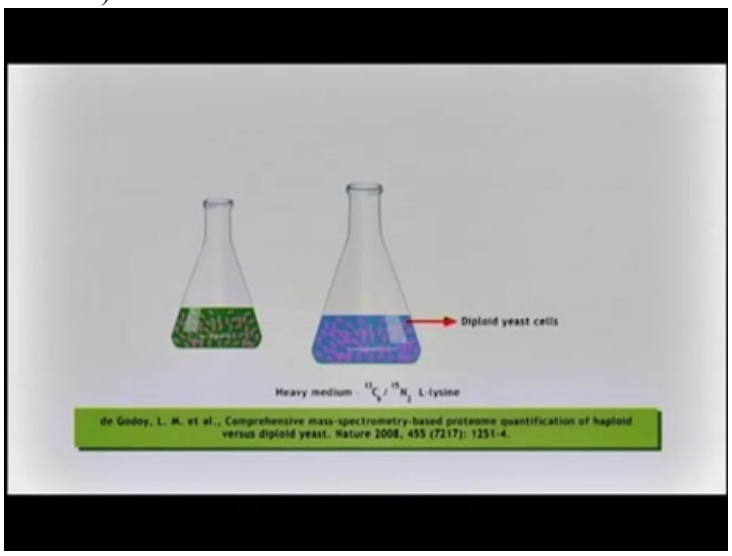

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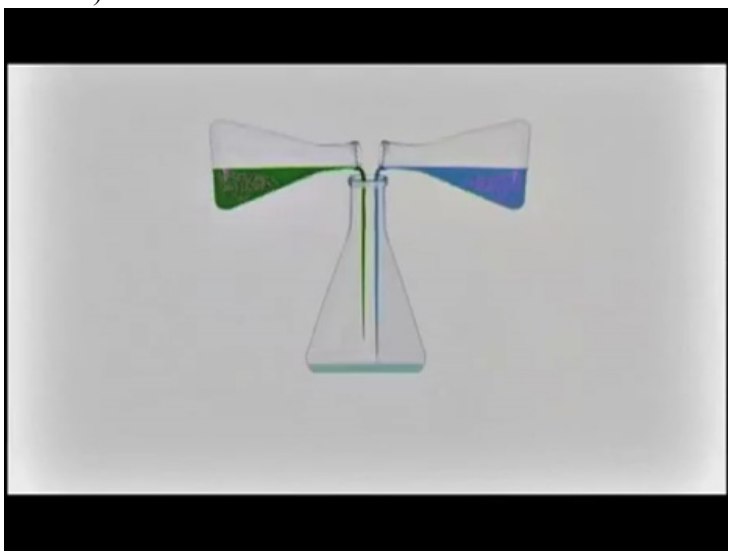

The cultures were mixed, proteins extracted and analyzed by LC-MS/MS.

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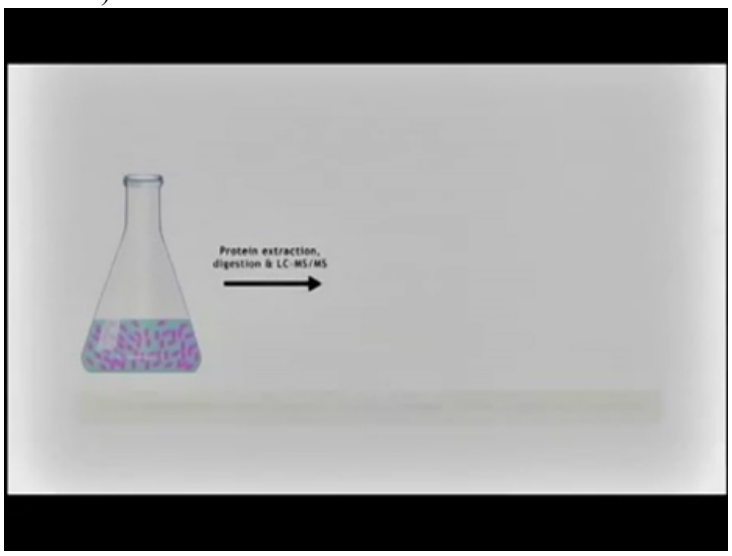

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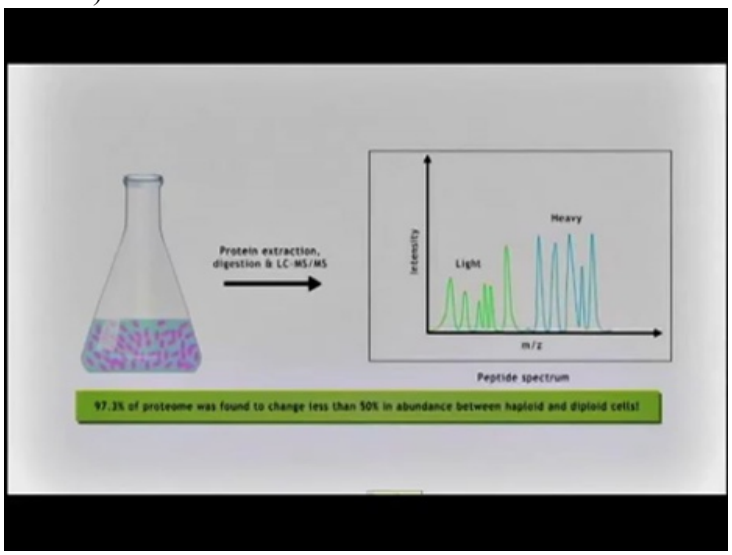

Protein ratios between haploid and diploid cells were determined with high accuracy. Comparison revealed 97.3% of the proteome changes less than 50% in abundance between haploid and diploid cells.

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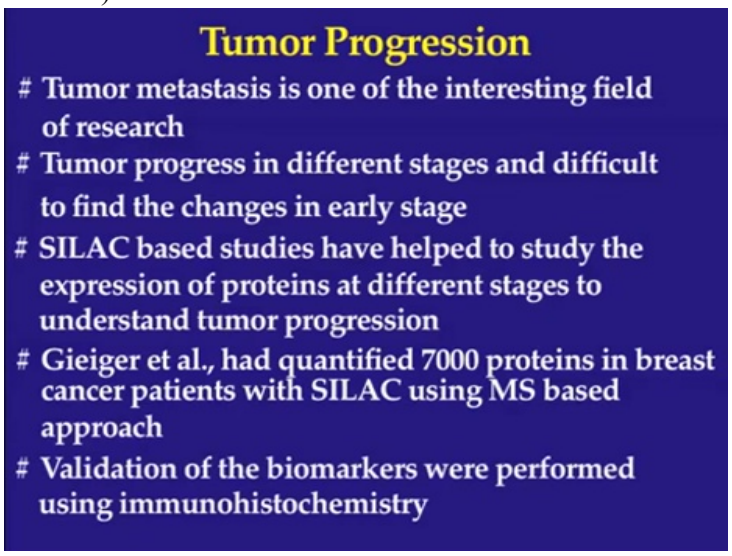

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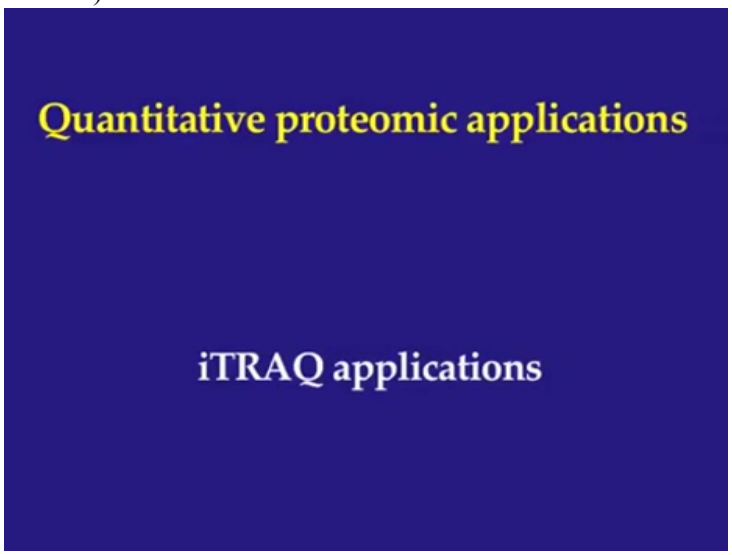

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Let us briefly look at the iTRAQ applications and I will show you this in this animation. In this animation we will look at one application of iTRAQ method.

A study performed by Boylan et al. in 2010 used iTRAQ for the identification of candidate biomarkers in ovarian cancer serum.

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Serum samples from l control and

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… cancer patients were first of all, depleted by using …

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…a multiple affinity removal system to carry out immune-depletion of serum samples from normal controls and ovarian cancer samples

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This step helped in removing the high abundance proteins, leaving behind only the medium and

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low abundance proteins

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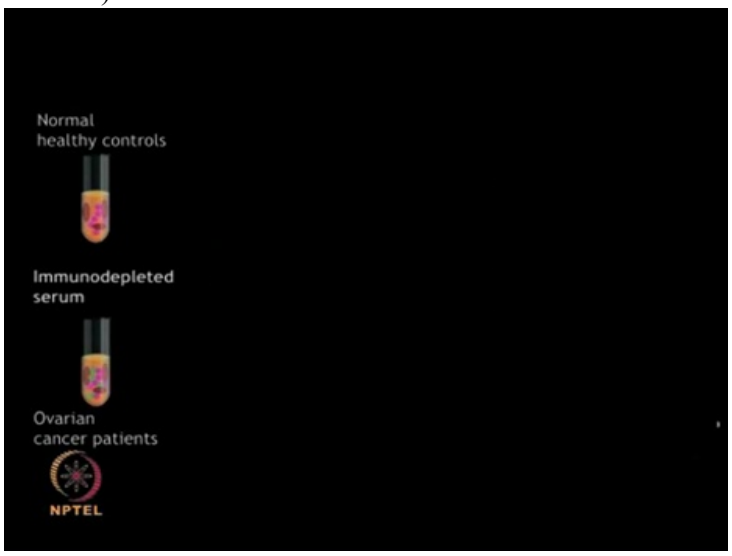

… for iTRAQ analysis

The immuno-depleted serum samples were then labeled with the iTRAQ reagents …

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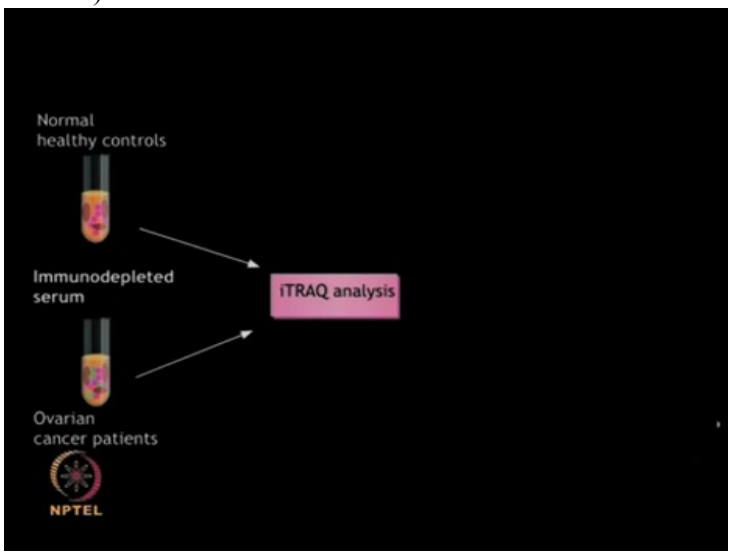

…and further analyzed…

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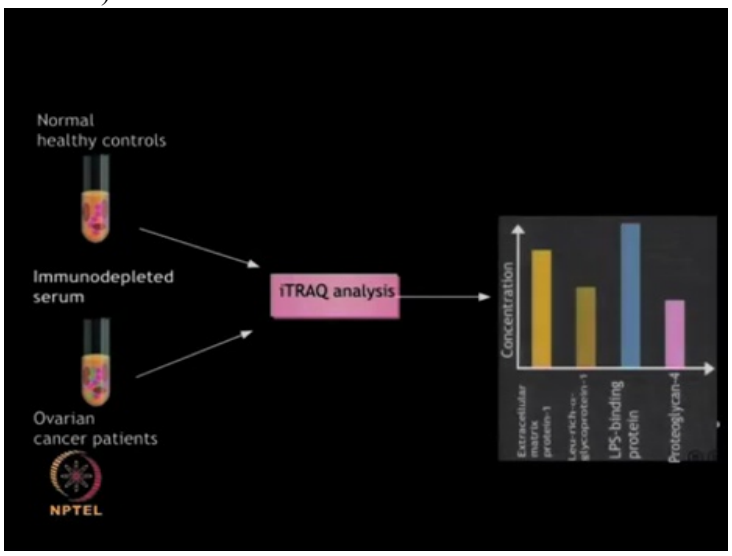

… in MS/MS

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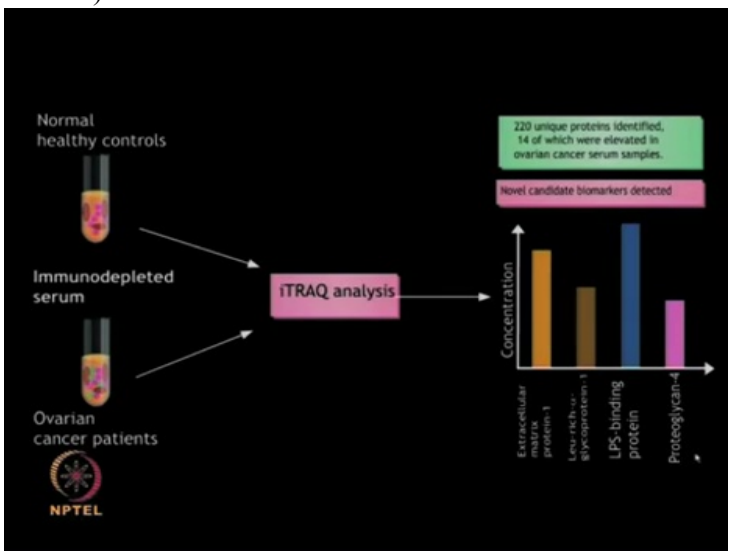

The authors detected a total of 220 unique proteins of which 14 were found to be elevated in the ovarian cancer serum samples as compared to the healthy controls and 4 novel candidate biomarkers were first time reported.

These results were further validated by Western immunoblotting. This just gives you an overview of how iTRAQ reagents can be used for various types of applications including biomarker discovery.

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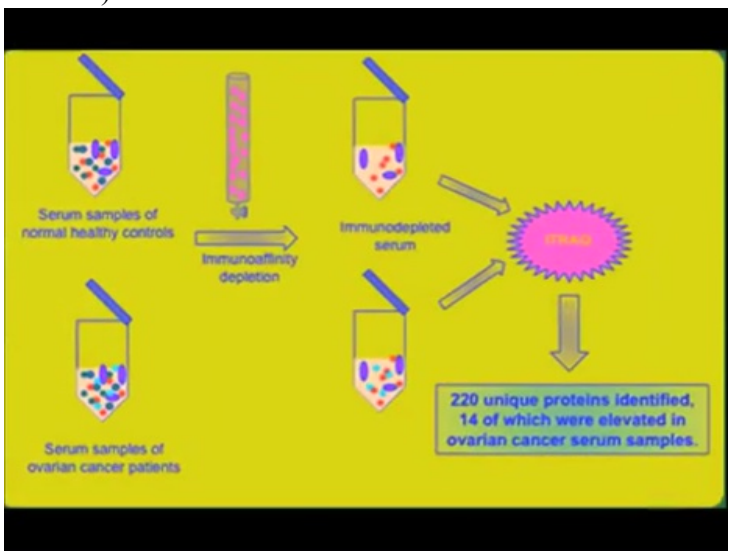

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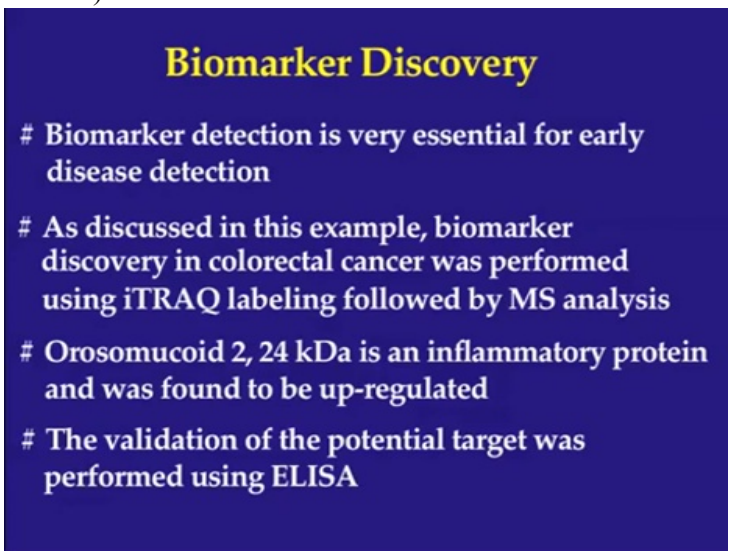

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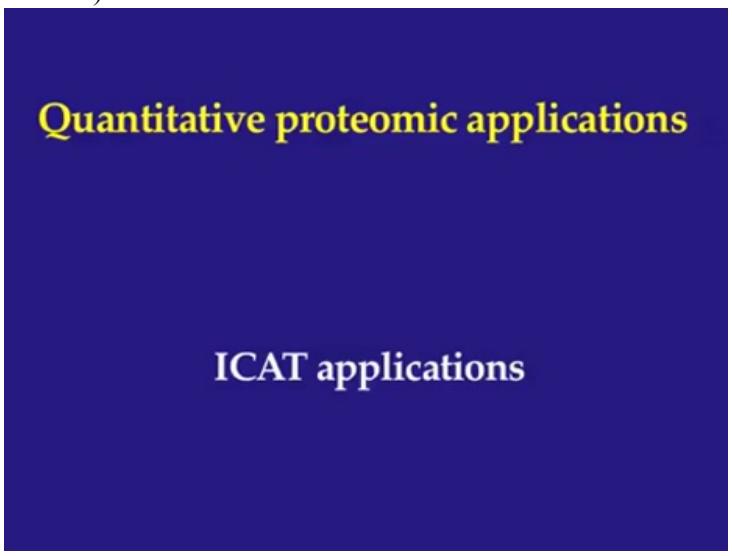

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ICAT has found many applications for proteomic studies. In one such clinical application, Kang and coworkers obtained …

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…plasma proteome from 6 breast cancer patients and 6 healthy controls.

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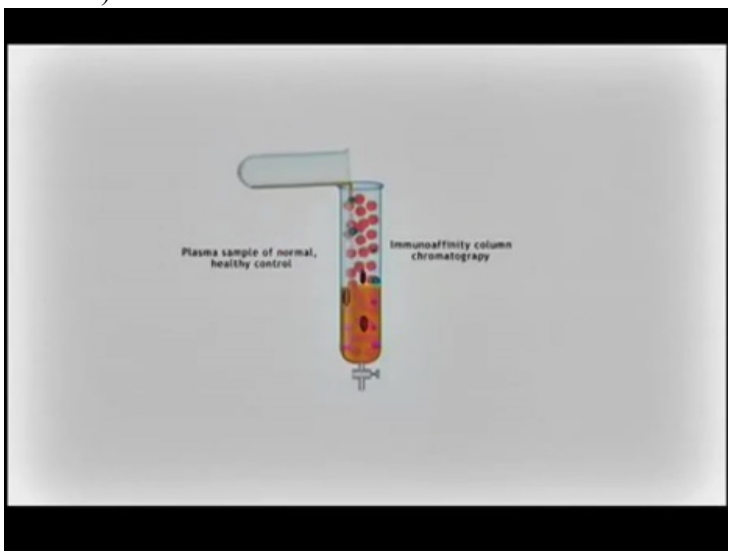

These plasma samples were first treated

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… on an immune affinity column …

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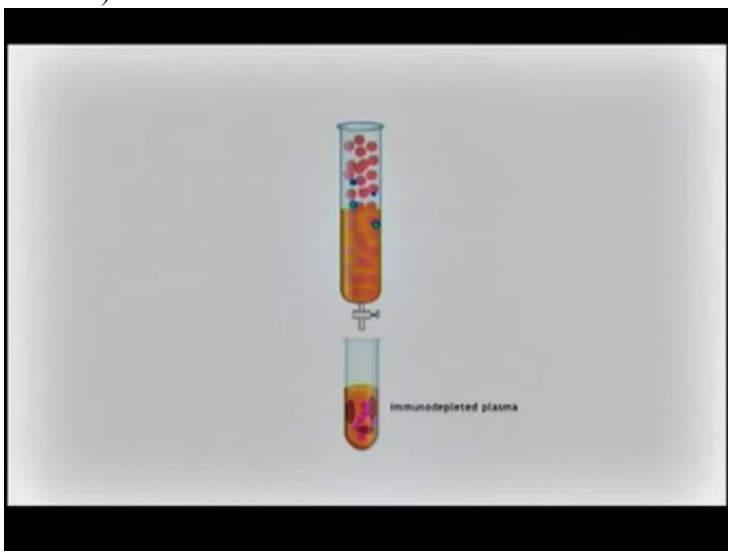

…in order to deplete them of their high abundance proteins

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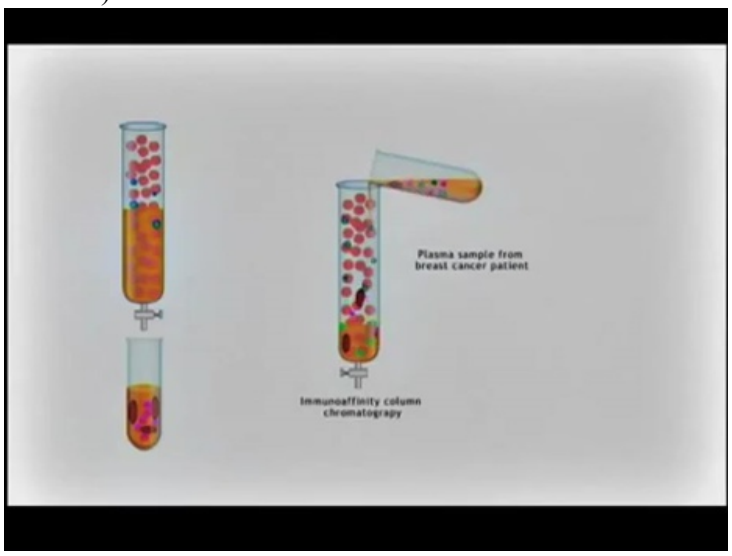

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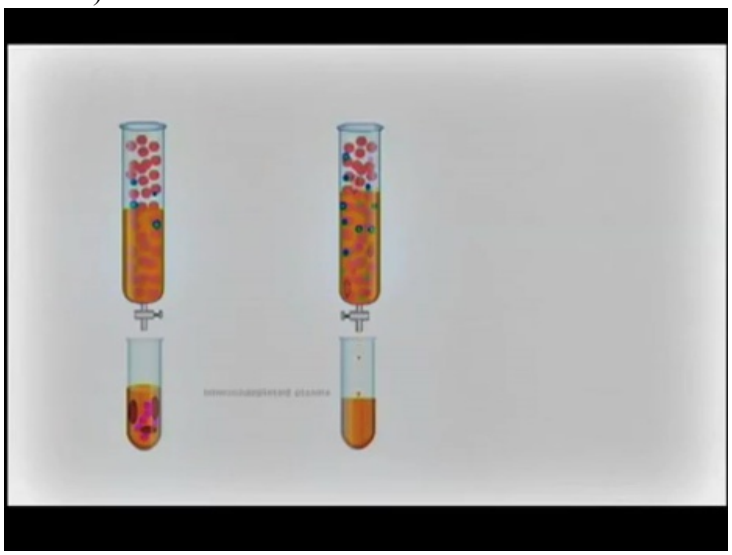

The immuno-depleted serum samples were then used for

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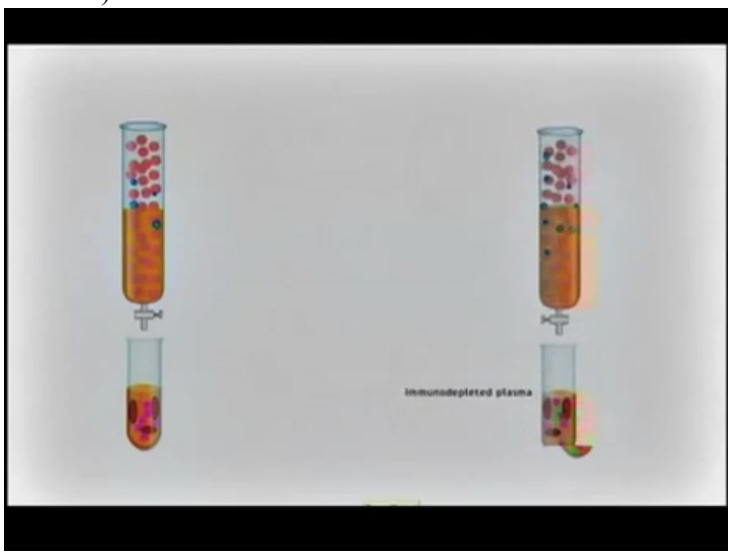

further analysis by ICAT.

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These samples were first treated on immune-affinity column

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…in order to deplete the of their high abundance proteins

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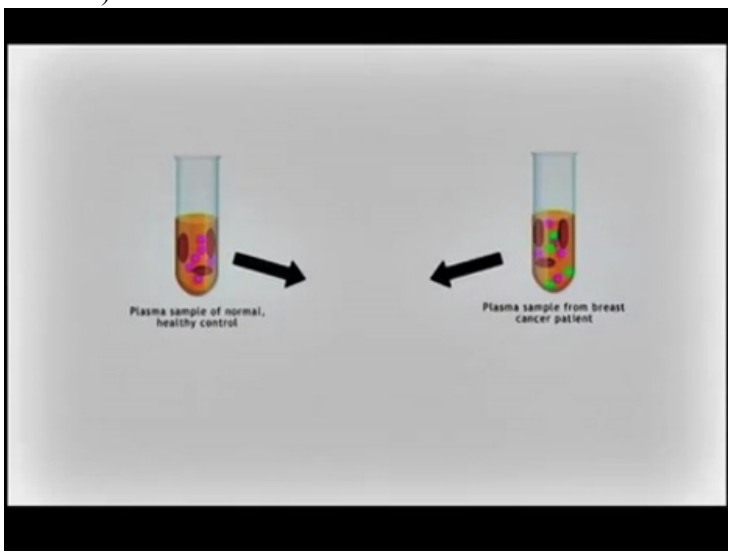

The immuno-depleted serum samples were then used …

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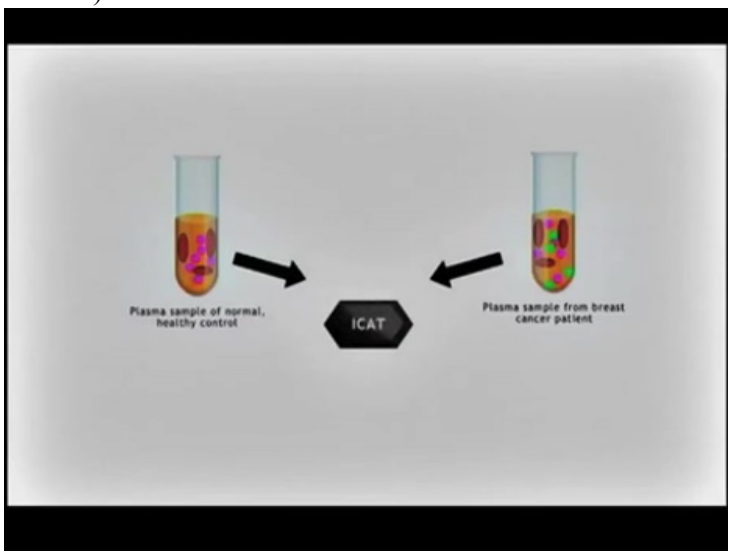

… for further analysis…

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…by ICAT. 155 proteins were identified…

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… of which 33 showed 1.5-fold abundance changes in plasma of breast cancer patients as compared with the healthy controls

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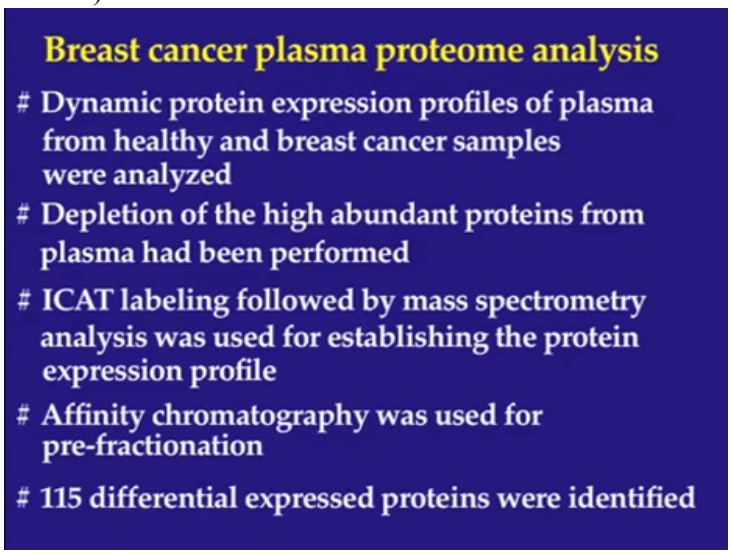

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In 2010, Human Proteome Organization has launched a global Human Proteome Project HPP. This project is designed to map the entire human proteins encoded by the genome.

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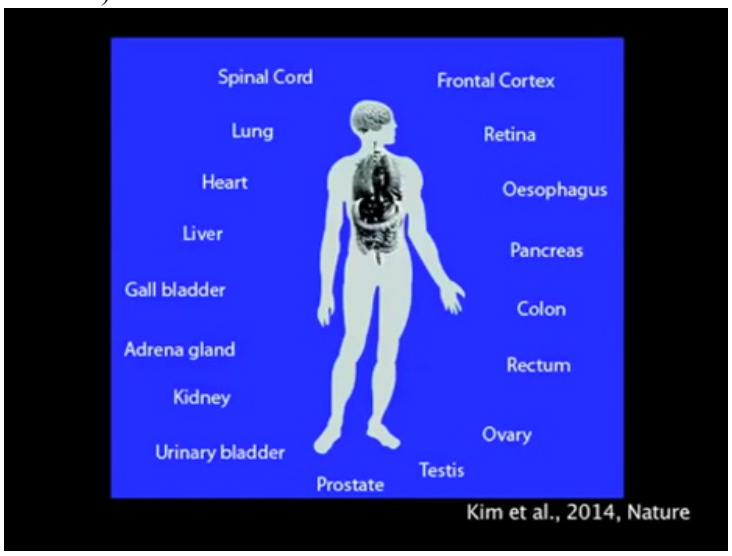

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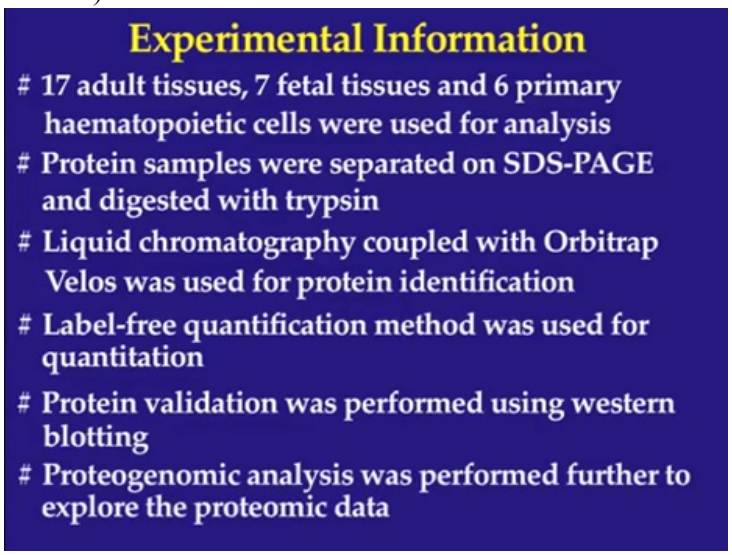

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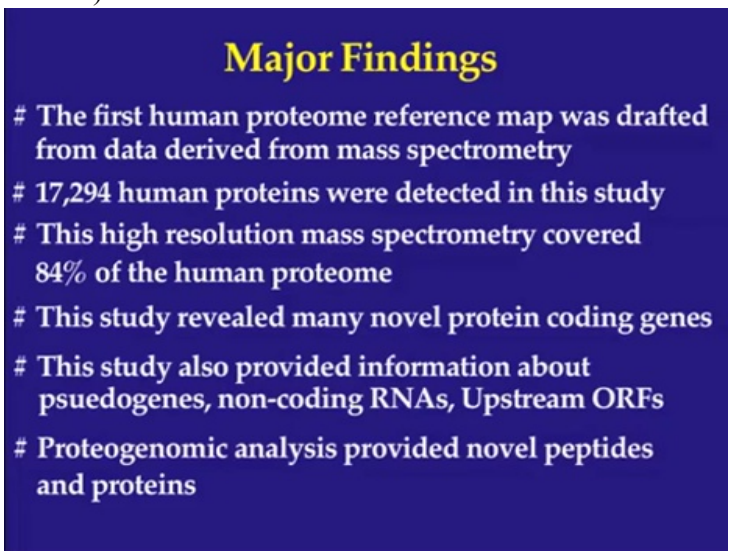

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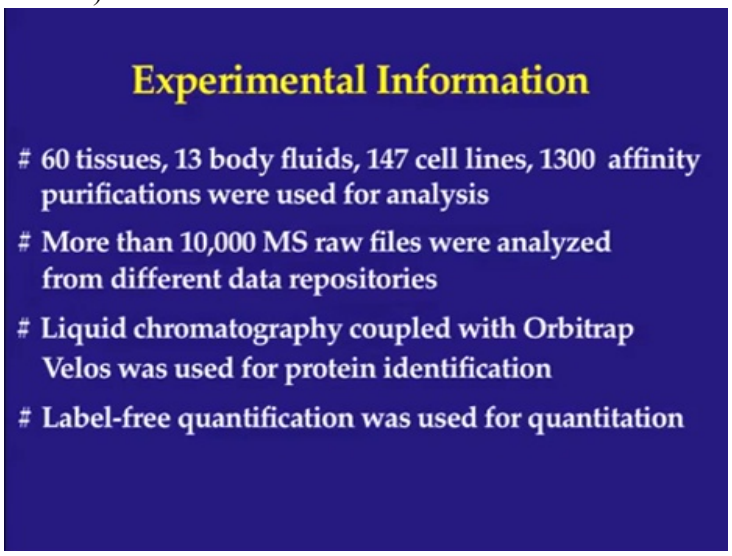

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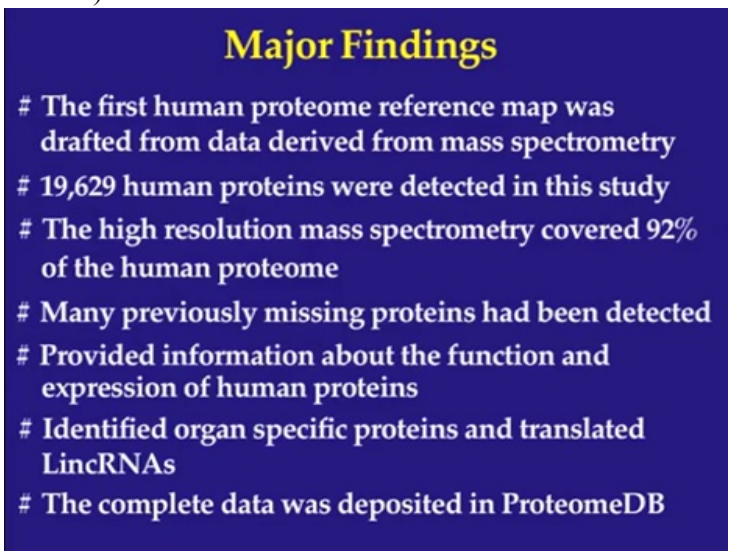

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Let us now discuss some of the targeted focus initiatives.

The Human Proteome Liver Project, this is the first initiative for Human Proteome Project for human organ, tissues with an intention of generation of comprehensive protein atlas of the liver and international liver tissue network, collection and distribution of normal liver samples and validation of new discoveries.

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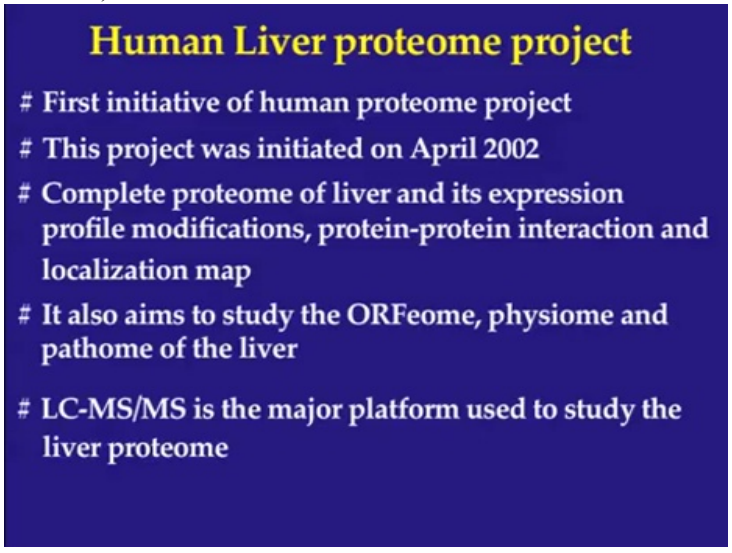

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Human Plasma Proteome Project, analysis of the protein constituents of human plasma and serum.

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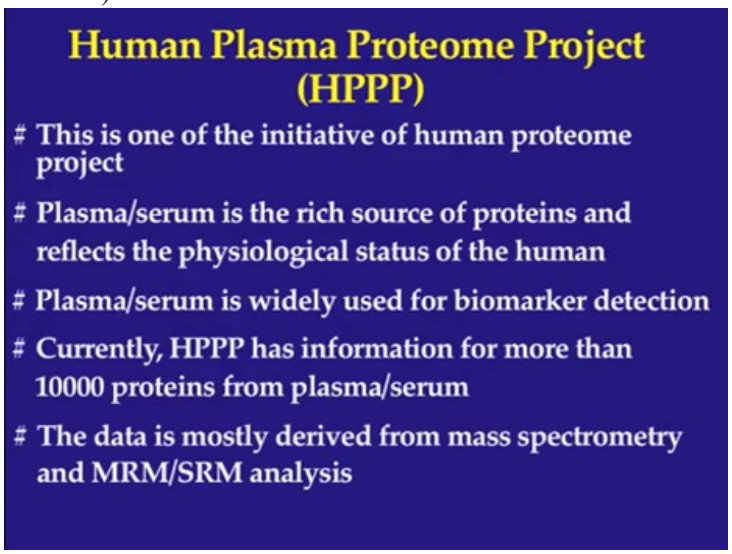

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Human Brain Proteome Project BPP focuses on the revolution of the brain related proteomics alteration, focusing on understanding neuro-degenerative diseases, aging and identification of prognostic and diagnostic biomarkers.

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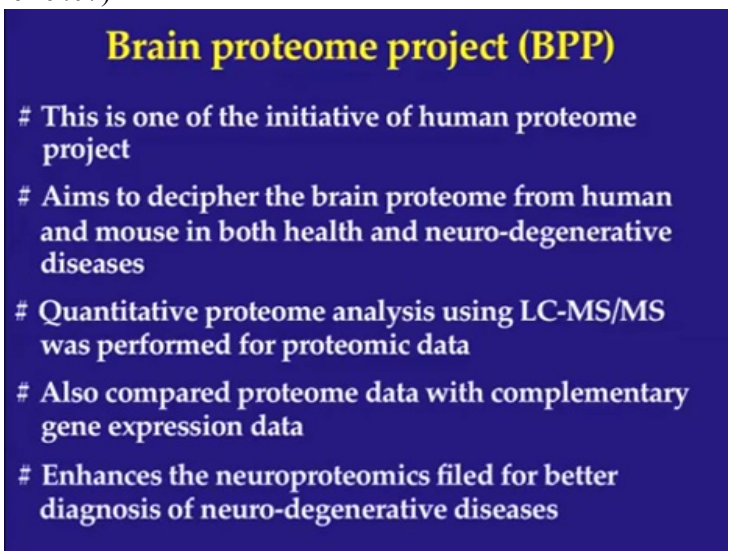

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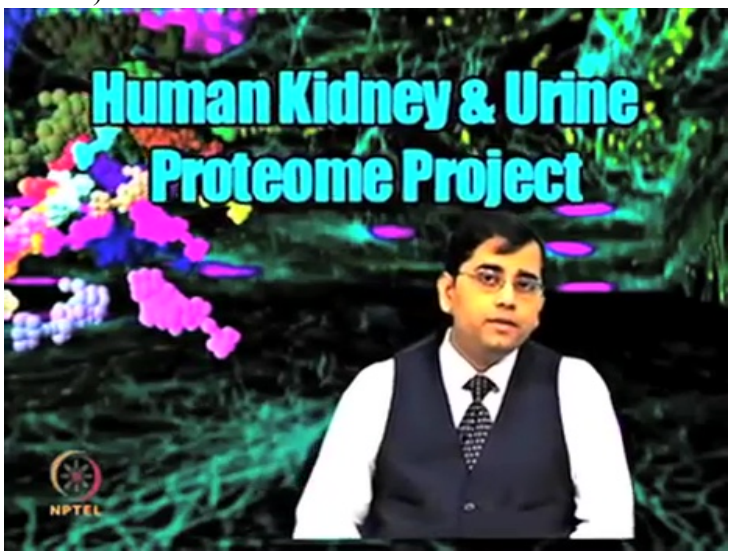

Human Kidney and Urine Proteome Project aims to understand kidney functions, mechanism of chronic kidney diseases at a protein level and discover biomarkers and target molecules for due therapeutics of kidney diseases.

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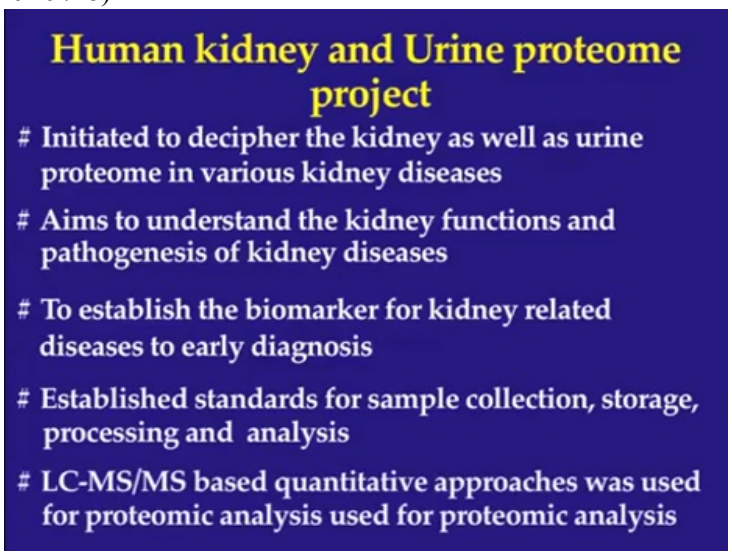

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