Discriminating sub-population responses of a mixture of human cell lines by proteogenomics

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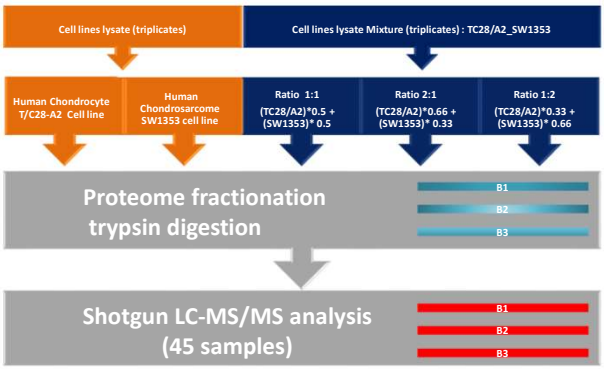
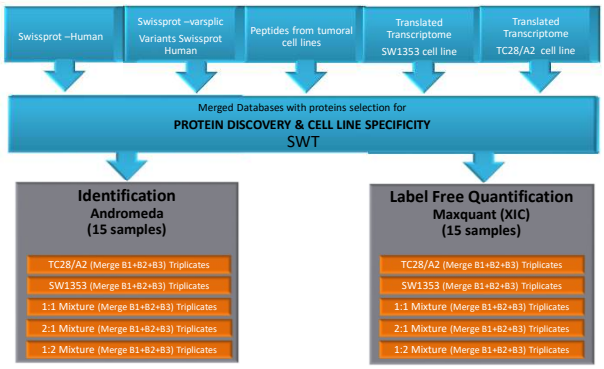
Monitoring proteome dynamics from different human cell types present concomitantly in a given sample is of great interest and could be applied to ultra-precise molecular characterization of complex tissues. Here, we propose a proteogenomics-based strategy to point at cell line molecular signatures. For this, the proteome is analyzed by high-throughput shotgun mass spectrometry and specific bioinformatics search are performed. First, mRNA from chondrosarcoma cells (SW1353 cell line) and immortalized chondrocytes (T/C28A2 cell line) were sequenced by RNAseq for establishing the most appropriate protein sequence database. For this an innovative cascade search allows to conciliate *de novo* and mapping RNAseq assemblies and the Human swissprot databases (Cogne et al., 2018). A set of 2 million of discriminating peptide sequences of the two cell lines are then identified. From them, 480 peptide sequences were detected and monitored based on extracted ion chromatogram (XIC) signals recorded by tandem mass spectrometry. A list of 55 peptides were used for quantitating the ratio of each cell type in a given co-culture sample with high precision selected with cell lines mixed at 2:1, 1:1; and 1:2 ratio. This new methodology was used to analyze the bystander effect generated by irradiated chondrosarcoma cells (SW1353 cell line) on immortalized chondrocytes (T/C28A2 cell line) in co-culture conditions. Such strategy could be applied to investigate intercellular interactions between different cell types, paving the way to new insights into the molecular mechanisms of crosstalk between human cells.

Samples preparation and analysis
Proteome fractionation for a large analysis

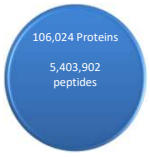


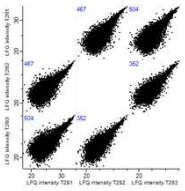
Database design and peptide quantification
Merge of Multi database for a large identification



output

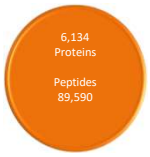
Database SWT

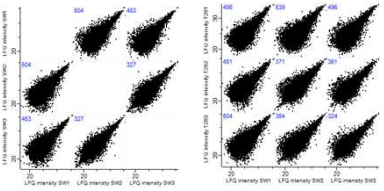




Euclidean distance between the two cell lines and their triplicates =>
No significant difference

Samples Analysis

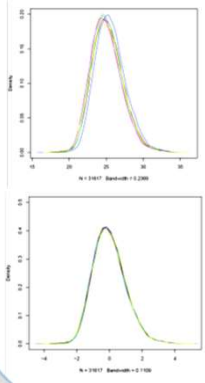


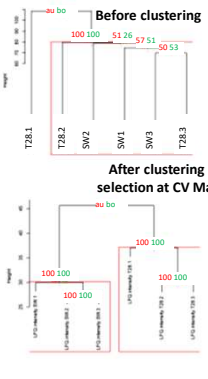


Search of discriminating peptides between the cell line SW1353 and TC28/A2

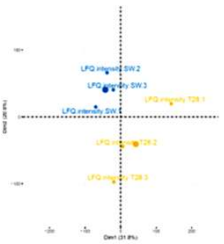
Scaling & Centering

hierarchical ascendant classification
Pvclust : method.dist="euclidean", method.hclust="ward.D2"

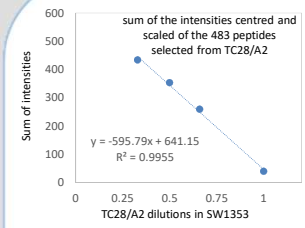




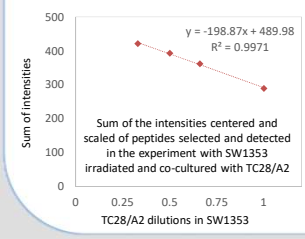
PCA after clustering and selection at CV Max 0.4



Results and conclusion



	Experiment 1					Experiment 2			
	Dilutions			No dilution	No dilution	Internal control	Mixture		Irradiation Control
	(TC28/A2)*0.33 + (SW1353)* 0.66	(TC28/A2)*0.5 + (SW1353)* 0.5	(TC28/A2)*0.66 + (SW1353)* 0.33	(TC28/A2) *1	(SW1353) *1	(TC28/A2)*0.5 + (SW1353)* 0.5 ctrl_0Gy	(TC28/A2)*0.5 + (SW1353)* 0.5 Irr_0.1Gy	(TC28/A2)*0.5 + (SW1353)* 0.5 Irr_2Gy	SW1353 Irr_0.1 Gy
Sum of the peptides intensities scaled & centered	421.10	393.07	361.44	289.14	287.76	230.80	233.72	196.08	221.57
Sum of the peptides intensities scaled, centered & normalized with experiment 1						393.07	398.03	333.94	377.34



	experiment 2		
	Mixture		Irradiation Control
Calculation of the TC28/A2 dilution with the linear model	0.46	0.78	1.31
comparison of the level expression SW_irr 0.1Gy/ SW			increase 30%
Result	(TC28/A2)*0.46 + (SW1353)* 0.54	(TC28/A2)*0.78 + (SW1353)* 0.22	

A new methodology based on proteogenomics approach including merged databases and extracted ion chromatogram (XIC) from selected peptides was developed for
1 =>discriminating two genetically closed cell lines in the mixture
2 =>evaluating in the mixture each cell line proportion
This method can be applied to experiments done with co-cultured cell lines.