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Center for Translational Molecular Medicine

PamGene kinase peptide array technology for identification of clinically relevant biomarkers in rheumatoid arthritis

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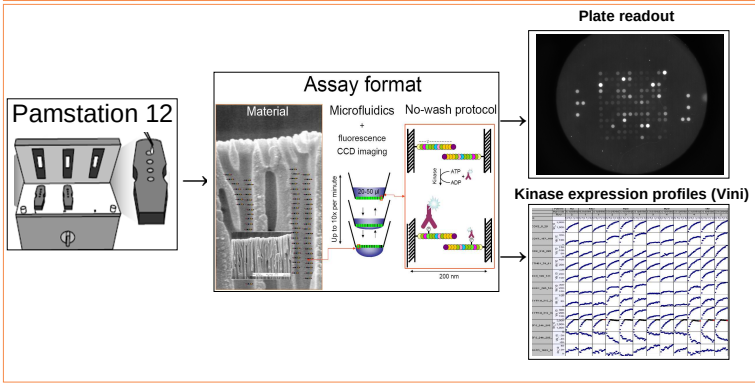
INTRODUCTION: Rheumatoid arthritis (RA) is one of the most prevalent systemic autoimmune disorders affecting about 1% of the population. Protein kinases are intracellular molecules that translate signals into cellular responses through signal transduction pathways, which play a central role in cell growth, cell proliferation, apoptosis and immune modulation. RA is a heterogeneous disease, thought to be the result of disperse signalling of protein kinase-mediated networks. In our search for clinical relevant biomarkers for various RA patients, we focus our research activities on kinase signalling networks that have diagnostic and/or prognostic value in RA. Thereto, we use the PamGene high-throughput microarray-based kinome profiling technology to monitor kinase activity in a multiplex setting using cell lysates of peripheral blood mononuclear cells (PBMCs) from RA patients and healthy controls. This method allows fast identification of tyrosine kinases that may provide insight in the deregulated kinase signalling network and has potential as clinical relevant biomarker .

AIM of STUDY: Set up of a work-flow protocol using the PamGene kinase peptide array technology for identification of relevant biomarkers in early RA patients (i.e. biomarker discovery).

TECHNOLOGY: Kinase activity of lysed PBMCs from 18 early RA patients and 6 healthy controls is determined using the PamGene protein micro-array containing144 unique and kinase specific 15 amino acid peptide tyrosine phosphorylation substrates. Phosphorylation is measured as the endpoint fluorescent signal emitted by the fluorescein-labeled anti-phosphotyrosine antibody (pY20) and calculated using the BioNavigator algorithm, resulting in kinase activity profiles.

RESULTS: PamGene protein micro-array technology was applied to measure tyrosine kinase activity in PBMC lysates from18 early RA patients (m-series) and 6 healthy controls (hc-series) according to the PamGene protocol (table 1). In each 4-well array a calibrator sample consisting of pooled lysates from 6 healthy controls (m8-ctrl series) was included. In total 8x4x3 (3 = triplicate) well arrays (A-H) were run. Subsequent data analyses were done on initial velocities (V_{ini}) using the Bionavigator algorithm. Based on these data we developed a data analysis flow protocol for calibration and normalization to allow cross-comparison of early RA and HC kinome data. Firstly, we calculated the median of the triplo's. Secondly, median values of the 4-well data were calibrated based on the m8-ctrl calibrator. Thirdly, the V_{ini} data were normalized (median centering, standardization, quantile normalization). Fourthly, data were analyzed using statistical tests. This workflow allowed us to identify kinases of diagnostic interest, which will be verified using kinase-specific customized assays.



EXPERIMENTAL SETUP

A	B	C	D	E	F	G	H
m3_1	m6_1	m12_1	m15_1	m22_1	m29_1	hc1_1	hc7_1
m3_2	m6_2	m12_2	m15_2	m22_2	m29_2	hc1_2	hc7_2
m3_3	m6_3	m12_3	m15_3	m22_3	m29_3	hc1_3	hc9_3
m4_1	m7_1	m13_1	m16_1	m25_1	m31_1	hc2_1	hc9_1
m4_2	m7_2	m13_2	m16_2	m25_2	m31_2	hc2_2	hc9_2
m4_3	m7_3	m13_3	m16_3	m25_3	m31_3	hc2_3	hc9_3
m5_1	m9_1	m14_1	m17_1	m26_1	m32_1	hc11_1	hc16_1
m5_2	m9_2	m14_2	m17_2	m26_2	m32_2	hc11_2	hc16_2
m5_3	m9_3	m14_3	m17_3	m26_3	m32_3	hc11_3	hc16_3
m8-ctrl1_1	m8-ctrl2_1	m8-ctrl3_1	m8-ctrl4_2	m8-ctrl5_1	m8-ctrl6_1	m8-ctrl7_1	m8-ctrl8_1
m8-ctrl1_2	m8-ctrl2_2	m8-ctrl3_2	m8-ctrl4_3	m8-ctrl5_2	m8-ctrl6_2	m8-ctrl7_2	m8-ctrl8_2
m8-ctrl1_3	m8-ctrl2_3	m8-ctrl3_3		m8-ctrl5_3		m8-ctrl7_3	m8-ctrl8_3

TABLE 1: Setup of series of different PamGene kinase array experiments. Each run consists of 3 patients (triplicate, m3-m32) and 1 pooled healthy control (HC, in triplicate, m8) or 3 individual HCs (triplicate, hc) and 1 pooled HC (triplicate, m8).

WORKFLOW DIAGRAM:

[1] *Vini data* : lysates of 18 EA patients, 8 pooled healthy controls, 6 individual controls. The increase in fluorescence is monitored for each of the 144 substrates → the slope of each phosphorylation curve is calculated → converted into the initial velocity → V_{ini} data

[2] *stabilization* : experiment is carried out in triplicate and the median is calculated

[3] *filtration* : artificial kinases, kinases with very high values and negative values are removed

[4] *calibration* : calibrate against pool of healthy volunteers (set value of pool of controls to value 1)

[5] *normalization* : calculating the median of the samples and the sample multiplication factors → perform : *median centering, standardization, quantile normalization*

[6] *analysis* : apply proper statistical tests (i.e. Student's T-test, Mann-Whitney test)

[7] obtain *results* → interpretation → verification using independent platform

CONCLUSION: - We have operational the technology and data-analysis workflow using the PamGene peptide array based on V_{ini} data to identify tyrosine kinases of interest for diagnostic and prognostic purposes in RA.

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Reumafonds

Reade
revalidatie | reumatologie

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