Tumor kinome profiling of early systemic disease dissemination in rectal cancer

– signaling mediated by PDGFR, VEGFR, and EPOR

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Marie Grøn Sælen[1,5], Kjersti Flatmark[1,2], Sigurd Folkvord[1], Rik de Wijn[3], Heidi Rasmussen[1], Øystein Fodstad[1,5], Anne Hansen Ree[4,5]

Departments of [1]Tumor Biology and [2]Surgical Oncology, Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway; [3]PamGene International B.V., 's-Hertogenbosch, The Netherlands; [4]Department of Oncology, Akershus University Hospital, Lørenskog, Norway; [5]Institute of Clinical Medicine, University of Oslo, Oslo, Norway

BACKGROUND AND OBJECTIVE

Adaptive cellular responses to tumor hypoxia involve activation of a range of kinase signaling pathways, among them angiogenic signaling, which is recognized as a central regulatory mechanism of metastasis formation in colorectal cancer.

This study aimed to examine tumor kinase activity related to angiogenesis and how this might correlate with early systemic disease dissemination.

PATIENTS AND METHODS

For 55 patients with locally advanced rectal cancer, bone marrow micrometastasis (BMM) status was determined by immunomagnetic selection of tumor cells in bone marrow, sampled at the time of diagnosis, and used as biomarker of systemic disease dissemination.

From baseline tumor biopsies, tyrosine kinase inhibition profiles were generated by tyrosine kinase substrate arrays after *ex vivo* addition of the tyrosine kinase inhibitor sunitinib.

Association between the tumor kinome and early systemic dissemination in terms of BMM status was studied.

CONCLUSION

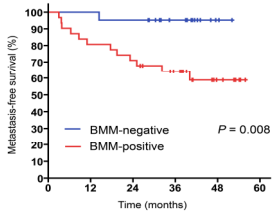
In this cohort of rectal cancer patients, a tumor phenotype defined by a subset of tyrosine kinase activities weakly inhibited by sunitinib *ex vivo*, particularly related to deficient PDGFR signaling, was associated with early systemic disease dissemination.

RESULTS – PATIENTS

Patient characteristics

	All patients (n = 55)	BMM-negative patients (n = 22)	BMM-positive patients (n = 33)
TNM stage			
T2	3 (6%)	2 (9%)	1 (3%)
T3	33 (60%)	14 (64%)	19 (58%)
T4	19 (35%)	6 (27%)	13 (39%)
N0	6 (11%)	2 (9%)	4 (12%)
N1	8 (15%)	3 (14%)	5 (15%)
N2	41 (75%)	17 (77%)	24 (72%)
M0	52 (95%)	21 (96%)	31 (94%)
M1	3 (6%)	1 (5%)	2 (6%)
Tumor regression grade			
1–2, good responders	40 (72%)	16 (73%)	24 (72%)
3, intermediate responders	9 (16%)	5 (23%)	4 (12%)
4, poor responders	6 (11%)	1 (5%)	5 (15%)
Gender			
Male	31 (56%)	13 (59%)	18 (55%)
Female	24 (43%)	9 (41%)	15 (46%)
Median age (range), years			
	61 (31–73)	61 (38–73)	59 (31–73)
Follow-up (median 41 months, range 7–58)			
Overt metastasis	16 (29%)	2 (10%)	14 (42%)
Death	8 (15%)	2 (10%)	6 (18%)

BMM-positive patients had poor metastasis-free survival



RESULTS – TUMOR TYROSINE KINASE ACTIVITY

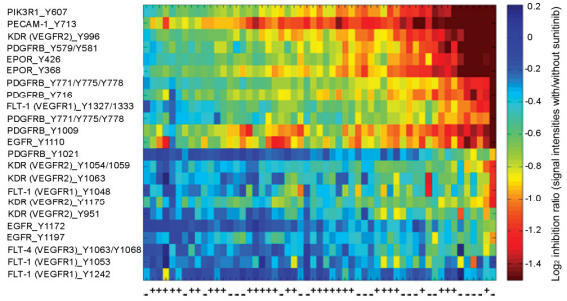
Differential *ex vivo* tumor kinase activity inhibition in patients with and without BMM

Sunitinib inhibition profiles could be assessed for 102 array substrates. Phosphorylation of 31 substrates was significantly more strongly inhibited in patients with negative BMM status than in BMM-positive patients.

Peptide substrate	Position of peptide sequence	Phosphorylation	Common name
Angiogenesis			
PDGFRB	1002-1014	Y1009	Beta platelet-derived growth factor receptor
PDGFRB	709-721	Y716	Beta platelet-derived growth factor receptor
PDGFRB	771-783	Y771, Y775, Y778	Beta platelet-derived growth factor receptor
PDGFRB	768-780	Y771, Y775, Y778	Beta platelet-derived growth factor receptor
PDGFRB	572-584	Y579, Y581	Beta platelet-derived growth factor receptor
FLT-1 (VEGFR1)	1326-1338	Y1327, Y1333	Vascular endothelial growth factor receptor 1
KDR (VEGFR2)	1168-1180	Y1175	Vascular endothelial growth factor receptor 2
KDR (VEGFR2)	989-1001	Y996	Vascular endothelial growth factor receptor 2
EPOR	361-373	Y368	Erythropoietin receptor
EPOR	419-431	Y426	Erythropoietin receptor
PECAM-1	706-718	Y713	Platelet endothelial cell adhesion molecule
PI3KR1	600-612	Y607	Phosphatidylinositol 3-kinase regulatory alpha subunit
EGFR	1190-1202	Y1197	Epidermal growth factor receptor
Cell adhesion, migration, and invasion			
CALM1	95-107	Y100	Calmodulin
FES	706-718	Y713	Proto-oncogene tyrosine-protein kinase Fes/Fps
FER	707-719	Y714	Proto-oncogene tyrosine-protein kinase FER
LCK	387-399	Y394	Proto-oncogene tyrosine-protein kinase LCK
PXN	111-123	Y118	Paxillin
PXN	24-36	Y31/33	Paxillin
MST1R	1353-1365	Y1353, Y1360	Macrophage-stimulating protein receptor
CTTN1	476-488	Y477, Y483	Src substrate protein p85
Cell survival and proliferation			
CTNNB1	79-91	Y86	Beta-catenin
JAK1	1015-1027	Y1022, Y1023	Tyrosine-protein kinase JAK1
PDPK1	2-14	Y9	3-phosphoinositide dependent protein kinase 1
Other			
CD247	116-128	Y123	T-cell surface glycoprotein CD3 zeta chain
CDK2	8-20	Y15, Y19	Cell division protein kinase 2
EPHA7	607-619	Y608, Y614	Ephrin type-A receptor 7
EPHB1	771-783	Y778	Ephrin type-B receptor 1
FRK	380-392	Y387	Tyrosine-protein kinase FRK
KRT6E	53-65	Y62	Keratin, type II cytoskeletal 6E
RET	1022-1034	Y1029	Proto-oncogene tyrosine-protein kinase receptor ret

Sunitinib inhibition profiles from angiogenesis-related kinase substrates

Within the 102-peptide panel, 23 angiogenesis-related substrates were identified. *Ex vivo* inhibition of tumor kinase activity was stronger in BMM-negative patients than in BMM-positive individuals ($P = 0.02$).



Patient tumor samples along horizontal axis, annotated by BMM status (+ positive, – negative), and phosphosubstrates along vertical axis. Red corresponds to stronger and blue to weaker inhibition of substrate phosphorylation.

Angiogenesis-related array substrates with stronger response to sunitinib inhibition in patients with negative BMM status than in BMM-positive patients:

- five of six PDGFR type β substrates
- three of ten VEGFR substrates
- both EPOR substrates

Strong *ex vivo* sunitinib inhibition of PDGFR array substrate phosphorylation in tumor samples from BMM-negative patients could reflect high pericyte signaling activity of mature tumor vessels that are less permeable for metastasizing cells.