Novel ELISA for the measurement of increased endostatin in mice with glomerulonephritis

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SUMMARY

1. For the detection of circulating endostatin in mouse/rat samples a sandwich ELISA was developed and validated.

- **Small sample volume** (1 µl)
- **Short incubation** time (2.5 hours)
- **Precision** (<10 % CV)
- **100% specificity**
- **High sensitivity** (detection limit 0.5 nmol/l)
- **Accuracy** (mean recovery 91-97%)
- **Dilution linearity** (mean recovery 88-109%)
- **Sample stability** (at least 4x freeze/thaw cycles)

2. The glomerulonephritis phenotype of ETV6/RUNX1 and BCL2 transgenic mice is accompanied with higher endostatin serum concentration, which seems to better reflect progression of kidney dysfunction than the established kidney biomarker blood urea nitrogen (BUN).

CONCLUSION

This highly specific, validated mouse/rat endostatin ELISA may be used for further investigation of the role of endostatin as biomarker in the field of renal disorders.

INTRODUCTION

Endostatin is a 20 kDa protein produced by proteolytic cleavage of collagen XVIII. It is one of the most potent endothelial cell-specific inhibitors of angiogenesis with influence on proliferation, migration and apoptosis. Altered expression and increased circulating endostatin are associated with impaired kidney function. Endostatin is discussed as one promising biomarker for the prognosis and/or diagnosis of kidney disease.

Vav-BCL2 transgenic mice ($BCL2^{tg}$) are prone to suffer from follicular lymphoma with age and can develop kidney disease, i.e. glomerulonephritis. A synergism between the ETV6/RUNX1 fusion product and BCL2 was identified by intercrossing single transgenic mice for BCL2 and ETV6/RUNX1. Double transgenic animals (E/R^{tg};$BCL2^{tg}$) displayed an accelerated development of immune complex glomerulonephritis.

METHODS

We have developed an immunoassay for the measurement of circulating mouse and rat endostatin in serum and plasma samples. An immobilized polyclonal antibody is used to capture endostatin which is subsequently detected with a labelled polyclonal anti-endostatin antibody.

The ELISA was completely validated according to ICH and EMEA standard quality guidelines.

Endostatin and blood urea nitrogen concentration were measured in mouse serum samples of three transgenic mice and controls.

MOUSE/RAT ENDOSTATIN ELISA CHARACTERISTICS

Typical standard curve of mouse/rat endostatin ELISA

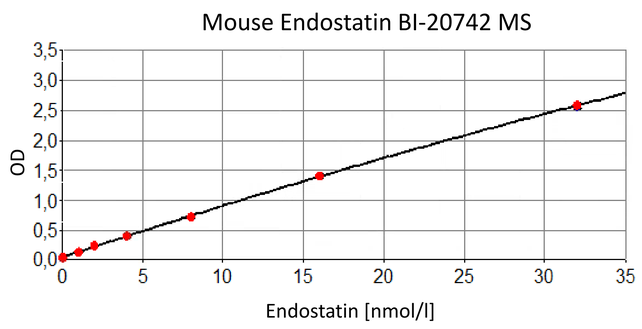

Fig. 1: Typical standard curve of mouse and rat endostatin ELISA with 7 standards and an assay range from 0-32 nmol/l.

Detection limit of the endostatin ELISA is 0.5 nmol/l

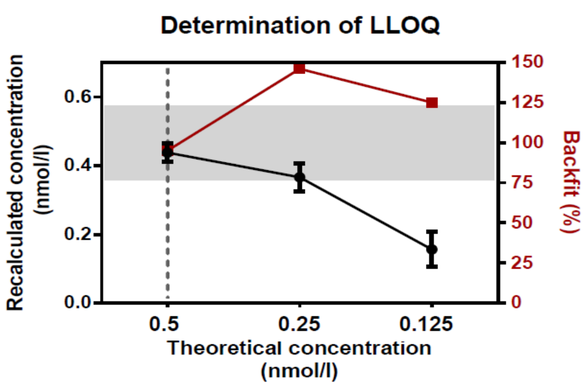

Fig. 2: For the **lower limit of quantification (LLOQ)** standard 2 was serially diluted. The backfit in % (red line) was calculated using the measured (recalculated) concentration (nmol/l; black y-axis) against theoretical concentration (nmol/l; x-axis) and should be within 75-125% (grey area).

The ELISA is highly specific for mouse and rat endostatin and shows good dilution linearity and accuracy

ASSAY PARAMETERS	Matrix (n)	Mean Recovery [%]
SPECIFICITY		
Competition	Serum Mouse (8)	100
	Plasma Mouse (8)	98
	Serum Rat (5)	100
DILUTION LINEARITY		
1:2	Serum Mouse (6)	101
	Plasma Mouse (5)	107
	Serum Rat (7)	96
1:4	Serum Mouse (6)	88
	Plasma Mouse (5)	109
	Serum Rat (7)	89
ACCURACY		
High Spike	Serum Mouse (7)	95
	Plasma Mouse (5)	91
	Serum Rat (4)	97
Low Spike	Serum Mouse (7)	95
	Plasma Mouse (5)	91
	Serum Rat (4)	97

Tab. 1: Mouse endostatin ELISA characteristics, whereas specificity was determined by adding at least 5-fold molar excess of coating antibody as competitor. For dilution linearity samples were diluted with assaybuffer. Accuracy was determined with spiking of two concentration (lower and upper range) of recombinant endostatin.

ENDOSTATIN AS A POTENTIAL BIOMARKER FOR IMPAIRED KIDNEY FUNCTION

$BCL2^{tg}$ and $ETV6/RUNX1^{tg}$;$BCL2^{tg}$ mice develop glomerulonephritis

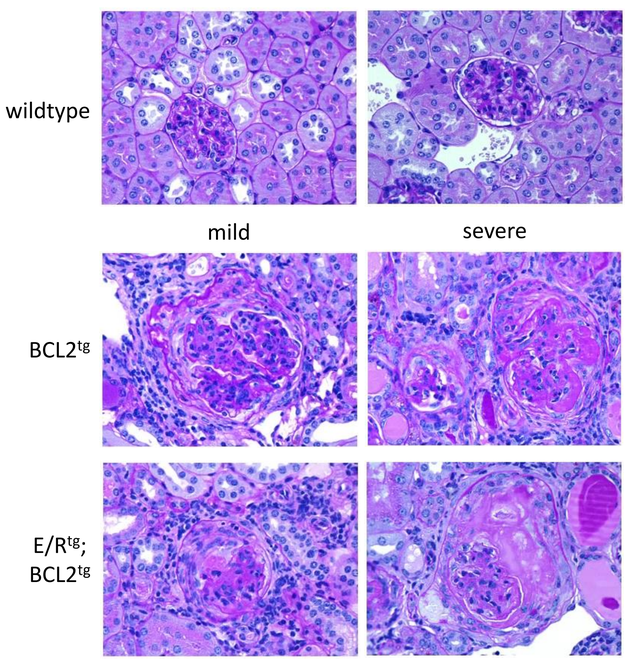

Fig. 3: Glomerulonephritis in transgenic mice
Representative pictures of kidney sections. Periodic acid Schiff (PAS) staining of the kidney sections indicates enlarged glomeruli and crescents in $BCL2^{tg}$ and E/R^{tg};$BCL2^{tg}$ transgenic mice.

Elevated endostatin levels better reflect kidney dysfunction within the genotypes

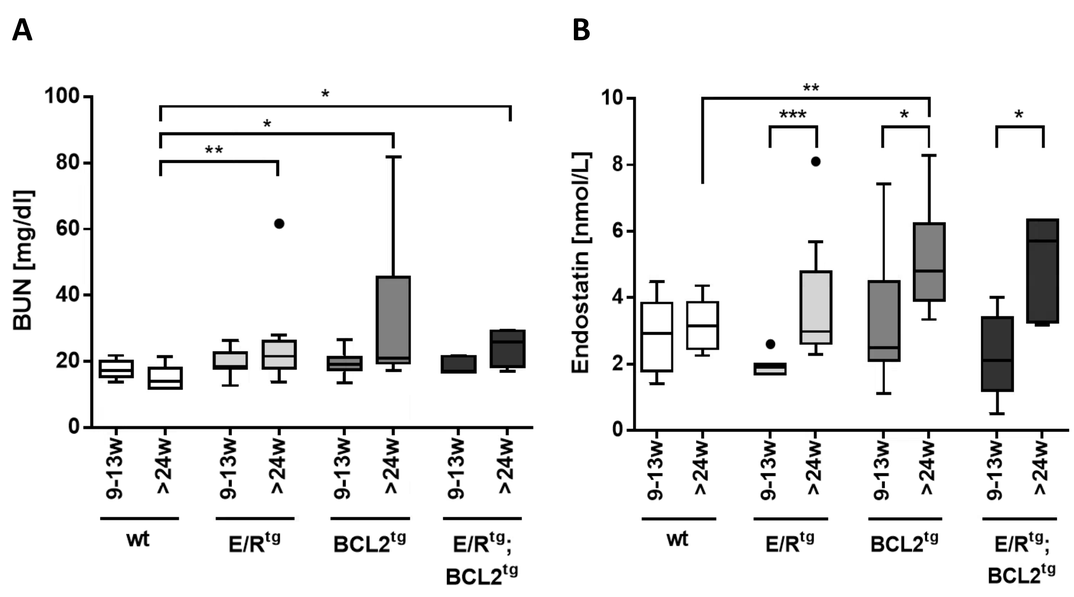

Fig. 4: BUN and endostatin levels in E/R^{tg}, $BCL2^{tg}$, $ETV6/RUNX1^{tg}$;$BCL2^{tg}$ mice
Tukey's boxplots indicate (A) blood urea nitrogen (BUN) levels (mg/dl) and (B) endostatin concentration (nmol/l) in serum of 9-13 weeks and more than 24 weeks old wild type (wt), E/R^{tg}, $BCL2^{tg}$ and $ETV6/RUNX1^{tg}$;$BCL2^{tg}$ mice. Significance with *** p<0.001, ** p<0.01 and * p<0.05.

LITERATURE

O'Reilly et al. (1997): Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 1997;88:277-85

Bauer et al. (2016): Cooperation of ETV6/RUNX1 and BCL2 enhances immunoglobulin production and accelerates glomerulonephritis in transgenic mice. Oncotarget 2016;7:12191-205

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Glomerulonephritis I

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