# Transcriptome analyses of zero kidney graft biopsies reveal a role of Metallothioneins in renal ageing

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## Background

Human life span is increasing continuously while structure and function of the kidney deteriorate with age. Age-related diseases contribute to this process and about one third of the population older than 70 years suffers from chronic kidney disease. Despite some similarities the aging kidney and CKD differ and it is unclear, whether the loss of renal function represents an intrinsic ageing process or is due to the accumulation of disease associated damage. Thus biological markers for age might be useful tools to dissect the specific pathologies and gene expression analysis of healthy aged kidneys might be a reasonable approach to avoid confounding by disease.

## METHODS

Age-associated gene expression changes in zero hour donor kidney biopsies were determined using microarray technology followed by ANOVA and SAM analysis. Expression changes of selected genes were confirmed by quantitative real-time PCR. *In situ* hybridization and immunohistochmistry was used to localize mRNA and protein expression in zero hour biopsies. Functional aspects were examined *in vitro* in RPTEC/TERT1 cells.

## RESULTS

Donors were classified into 3 age groups (<40, 40-59, >60 years). In Microarray data age-associated transcriptional changes were identified: 16 transcripts were found to be significantly upregulated in age group 3 as compared to age group 1. 8 of these transcripts encoded for metallothionein (MT) isoforms. All MT isoforms correlated significantly with 3 months creatinine values at 3 months but not later time points of follow-up. *In situ* hybridization demonstrated localization of MT mRNA in renal proximal tubular cells. RPTEC/TERT1 cells overexpressing MT2a were less susceptible towards hypoxia induced cytotoxicity.

| PROBE | SYMBOL | DESCRIPTION | Gr1 mean expr | Gr2 mean expr | Gr3 mean expr | p-value of group 1 vs group 3 |
|---|---|---|---|---|---|---|
| A_32_P200144 | IGHG1 | immunoglobulin heavy constant gamma 1 | 6.76 | 8.60 | 8.97 | 0.024 |
| A_23_P43979 | IGLL5 | immunoglobulin lambda-like polypeptide 5 | 7.13 | 8.80 | 9.18 | 0.023 |
| A_23_P54840 | MT1A | metallothionein 1A | 10.83 | 11.85 | 12.30 | 0.003 |
| A_23_P37983 | MT1B | metallothionein 1B | 10.55 | 11.76 | 12.19 | 0.005 |
| A_23_P206724 | MT1E | metallothionein 1E | 10.67 | 11.93 | 12.42 | 0.005 |
| A_23_P206707 | MT1G | metallothionein 1G | 9.31 | 10.34 | 10.80 | 0.009 |
| A_23_P60933 | MT1G | metallothionein 1G | 11.39 | 12.63 | 13.04 | 0.013 |
| A_23_P414343 | MT1H | metallothionein 1H | 10.80 | 11.98 | 12.50 | 0.006 |
| A_23_P427703 | MT1L | metallothionein 1L (gene/pseudogene) | 10.52 | 11.80 | 12.26 | 0.009 |
| A_23_P66241 | MT1M | metallothionein 1M | 9.14 | 10.21 | 10.69 | 0.038 |
| A_23_P303242 | MT1X | metallothionein 1X | 10.82 | 11.96 | 12.48 | 0.002 |
| A_24_P125096 | MT1X | metallothionein 1X | 10.27 | 11.38 | 11.86 | 0.006 |
| A_23_P106844 | MT2A | metallothionein 2A | 11.96 | 13.01 | 13.48 | 0.024 |
| A_24_P361896 | MT2A | metallothionein 2A | 11.65 | 12.81 | 13.33 | 0.022 |
| A_23_P163782 | NA | NA | 10.33 | 11.45 | 11.91 | 0.016 |
| A_23_P252413 | NA | NA | 11.46 | 12.54 | 13.03 | 0.005 |

**Fig. 1** Microarray analysis revealed the following 16 transcripts to be significantly up-regulated in age group 3 (>59) as compared to age group 1 (<40). Data of the three groups indicate normalized intensity values in log2 scale. p for trend < 0.05 for all transcripts.

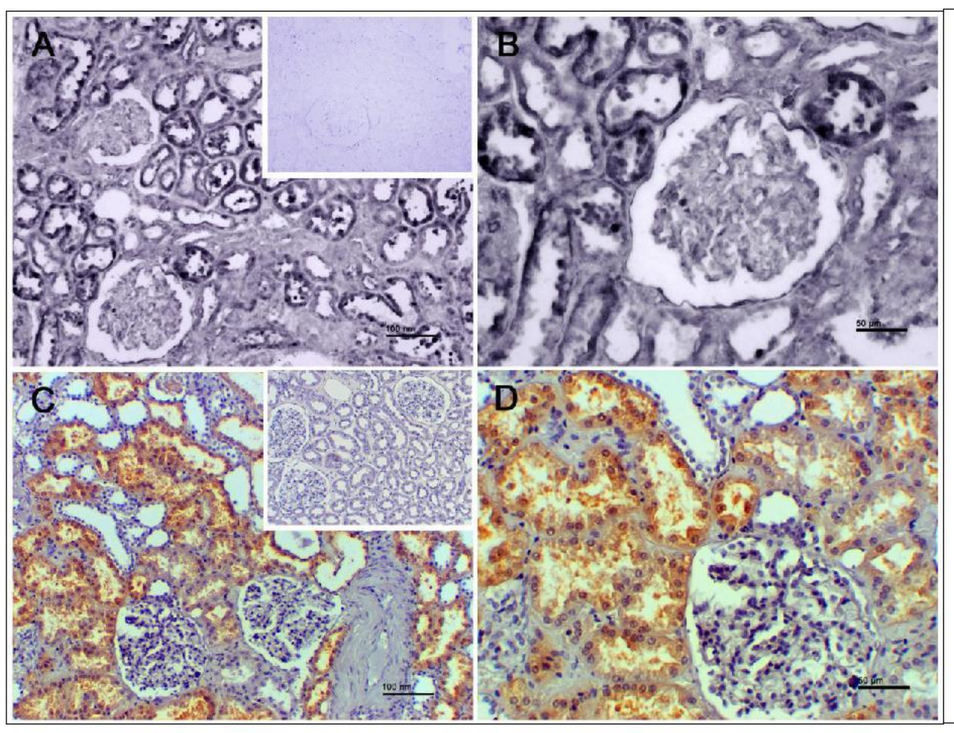
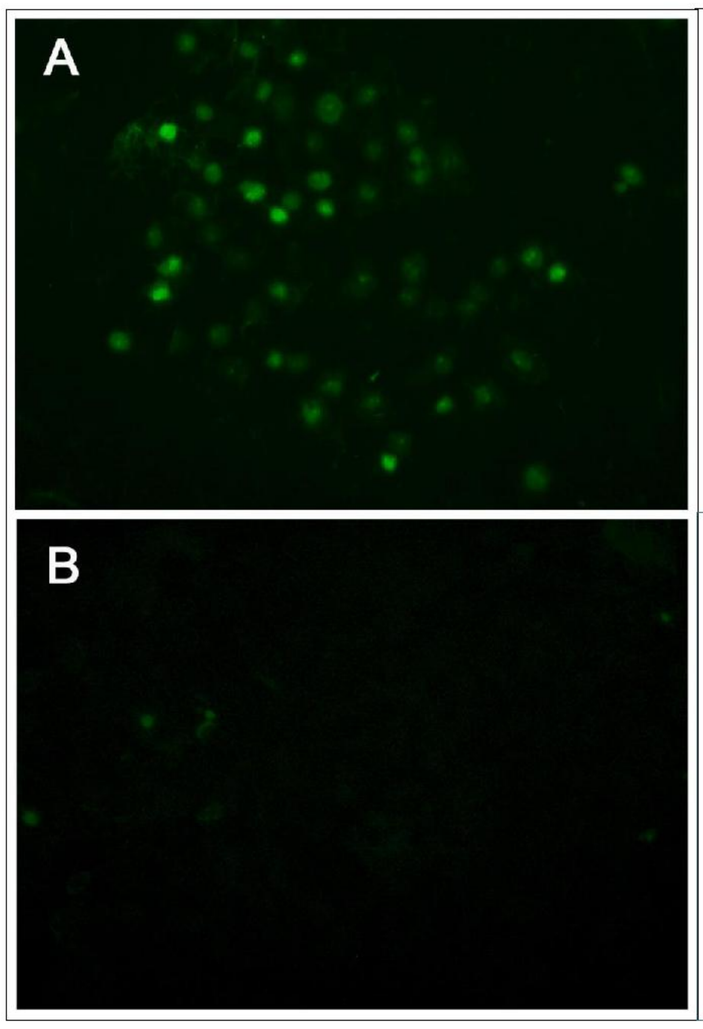



**Fig. 2** Representing images of in situ hybridization for MT2A mRNA (A,B) & immuno-histochemistry with an antibody against MT1 and MT2 isoforms (C,D). MT2A on the mRNA level (dark blue/black labels in A,B) & MTs on protein level (brown colored in C,D) are located in (epithelial cells of) proximal tubuli. Scale bares indicate 100 µm (A,C) & 50 µm (B,D).



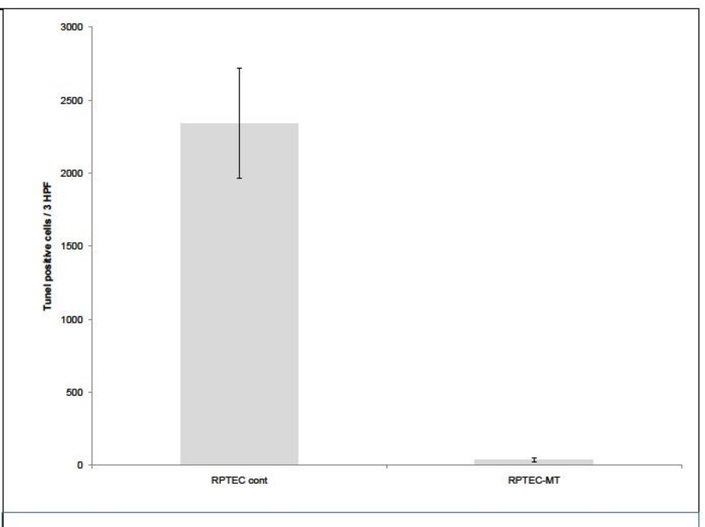




**Fig. 3** Effect of 36 hours of hypoxia on RPTEC control (A) and RPTEC MT (B) cells assessed by TUNEL assay. RPTEC MT cells were significantly (p<0,0001) less susceptible to hypoxia as control cells. Representing experiment of three with n=5 for each is shown.

## CONCLUSIONS

In our study MT isoforms are significantly upregulated with age. MT2A mRNA as well as MT1 and MT2 protein abundance, which showed the most significant changes associated with age, were mostly localized to the proximal renal tubules and their brush borders. MT2A overexpressing RPTECs were significantly less sustainable to induced ROS stress.

Metallothioneins (MT) play a role in the ageing kidney. As MTs contribute to detoxification of heavy metals and homeostasis of essential metals, protect from ROS mediated oxidative stress and prevent apoptosis their upregulation with ageing might represent an intrinsic protective mechanism.

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