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Decrease in LDL Receptor-Related Protein Expression and Function Correlates With Advanced Stages of Wilms Tumors

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Background. The molecular processes responsible for the invasive phenotype of pediatric Wilms tumors (WT) are poorly understood. A candidate WT suppressor gene (*WT1*) has been found mutated in a number of these pediatric kidney tumors. However, the disruption of normal WT1 protein function cannot solely explain WT growth. The aim of the present study is to identify new molecular players that regulate the invasive character of WT. ***Procedure.*** Fresh frozen samples from 45 renal tumors of Wilms were obtained from the National Wilms Tumor Study Group's Biological Samples Bank. Gelatin zymography, Western blotting, and immunodetection were used to compare tissue biopsies originating from the infiltrating (stage III), metastatic (stage IV), and anaplastic phenotype of Wilms' tumors (WT). ***Results.*** The expression of the low-density lipoprotein receptor-related protein (LRP) diminished in stage IV and anaplastic WT. Moreover, the expression of RAP, an LRP intracellular chaperone, was also decreased. The diminished expression of LRP and RAP correlated with increased levels of several known extracellular ligands that LRP usually recycles from the extracellular matrix (ECM) environment, including PAI-1, MMP-9, and TIMP-1. The proteolytic processing of MT1-MMP, a functional regulator of LRP, also correlated with the WT invasive phenotype. ***Conclusions.*** The low expression of LRP, whose function is regulated by MT1-MMP and whose activity in recycling ECM-associated proteolytic enzymes becomes drastically diminished in advanced stages of WT, may in part explain the acquired invasive potential of the developing WT pediatric cancer. Pediatr Blood Cancer 2006;46:40–49. © 2005 Wiley-Liss, Inc.

Key words: low-density lipoprotein receptor-related protein; membrane type-1 matrix metalloproteinase; Wilms' tumor

INTRODUCTION

Wilms tumors (WT), or nephroblastoma, are the second most common extracranial solid tumors of childhood, occurring in 1 in 10,000 children and accounting for approximately 8% of childhood cancers [1]. It is believed to result from malignant transformation of abnormally persistent renal stem cells which retain embryonic differentiation potential and which fail to undergo the normal maturation process [2–4]. Several studies indicate that the *WT1* gene is, so far, the only cloned gene involved in the occurrence of WT [5]. The *WT1* gene has been classified as a tumor suppressor gene and found to play an important role at different stages of kidney development [6,7]. Disruption of the WT1 protein, a zinc finger transcription factor which acts as a transcriptional repressor which is inactivated in a subset of WT cases [7,8], may thus lead to a whole spectrum of kidney diseases ranging from tumor development to mild forms of renal failure [9]. Accordingly, a hypothesis for WT pathogenesis states that loss of the negative regulator role of WT1 results in overexpression of one or more growth-promoting target genes [10]. Paradoxically, the disruption of normal WT1 function alone is not sufficient to cause WT [11], confirming that WT cancer biology is a multifactorial event. Moreover, conflicting correlations between the expression of p53 in WT makes its use as an independent prognostic factor difficult [12,13]. As, the invasive phenotype of the infiltrating (stage III), metastatic (stage IV) and anaplastic WT is poorly characterized, the identification of other molecular players that are involved in the pathogenesis and in the setting of the invasive phenotype of WT is thus required.

Profiling of differential gene expression in WT by cDNA expression array was recently used to identify new genes or pathways involved in WT etiology [14]. Interestingly, among the differentially expressed genes identified, the expression of a gene encoding membrane type-1 matrix metalloproteinase (MT1-MMP) was consistently found increased in WT. It is well known that MT1-MMP is involved in invasion and metastasis of gastric and colorectal cancers [15], as well as in the advanced stages of brain tumors [16]. The classical role of

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MT1-MMP is to proteolytically activate proMMP-2, which is then directly responsible for extracellular matrix (ECM) degradation [17]. During cancer invasion and angiogenesis, MT1-MMP also functions in endothelial tubulogenesis [18], in fibrinolysis [19], and in the regulation of the hyaluronic acid cell surface receptor CD44 [20]. Very recently, active MT1-MMP was also shown to up-regulate VEGF an angiogenic factor expressed in WT [21,22]. Collectively, these pleiotropic functions of MT1-MMP make this protein a potentially important therapeutic target.

ECM degradation/regulation by MMP thus represent events that need to be tightly regulated and which have important implications for many pathophysiological processes including pericellular proteolysis in neoplastic cells. Most recently, the low density lipoprotein receptor-related protein (LRP) was demonstrated to regulate the fate of soluble matrix-degrading proteases such as MMP-2 and MMP-9, and to be regulated by MT1-MMP proteolysis in malignant cells [23]. LRP recognizes at least 30 different ligands, including lipoproteins, proteinases, proteinase–inhibitor complexes, ECM proteins, bacterial toxins, viruses, and various intracellular proteins [24]. By far, the largest groups of ligands that are recognized by LRP are either proteinases or associated proteins regulating their proteolytic activity. LRP level and activity are known to be substantially decreased in tumors, which would decrease the catabolism of MMP-9 and other proteinases, leading to higher levels of these enzymes at tumor sites [25]. In agreement with this, constitutive activation of MMP-9 expression was recently shown in a WT cell line [26], which suggests that the ability of LRP to modulate the levels of soluble MMPs may play a major role in removing the excessive extracellular proteolytic activities potentially associated with the WT invasive and metastatic phenotypes.

In the present study, we assessed the specific protein expression levels of LRP, RAP, MMP-2, and -9, tissue inhibitor of MMP- (TIMP)-1, -2, and -4, and MT1-MMP in samples of WT. These samples represent the full clinical spectrum of favourable histology stages I-IV, and unfavourable or anaplastic histology, and were compared to levels in normal infant kidney. Our results highlight a crucial role for LRP-mediated functions in recycling ECM proteolytic enzymes and potential regulation by MT1-MMP that might characterize the invasive phenotype of stages III and IV as well as anaplastic WT.

MATERIALS AND METHODS

Materials

Bovine serum albumin (BSA), Triton X-100, and Tween-20 were purchased from Sigma (Oakville, ON). The anti-MT1-MMP polyclonal antibody AB-815, the rabbit polyclonal antibody against TIMP-1, TIMP-2, and TIMP-4 were from Chemicon (Temecula, CA). The mouse monoclonal antibody against the LRP β-subunit (85 kDa) was purchased from Research Diagnostics (Flanders, NJ). The mouse monoclonal RAP antibody was from Progen Immuno-Diagnostica. The monoclonal antibody against β-actin was from Sigma (St Louis, MO). The mouse and rabbit secondary antibodies were from Jackson ImmunoResearch Laboratory (West Grove, PA).

Tumors

Fresh frozen samples from 45 renal tumors of Wilms, comprising 9 specimens with favourable histology from each stage (I–IV), nine specimens with anaplastic histology from various stages and seven samples of normal infant kidney were obtained from the National Wilms' Tumor Study Group's Biological Samples Bank. The staging system and histological categorization employed was described by Green et al. [1]. Fetal kidney samples, that would have represented the likely cells of origin of WT, were unfortunately not available at time of analysis. Thus, the normal infant kidneys were used as control samples for all our immunodetection analysis. The respective tissues were weighed and homogenized in 5 volumes of buffer containing 250 mM sucrose and 10 mM Hepes/Tris pH 7.4, with a Polytron (Brinkmann Instruments, Roxdale, ON). Protein content was determined with the Coomassie Plus Protein Assay Reagent (Pierce, Rockford, IL) using BSA as a standard. Samples were aliquoted and frozen at -80°C until use.

Immunoblotting Procedures

Proteins from tissue homogenates were separated by SDS–polyacrylamide gel electrophoresis (PAGE). After electrophoresis, proteins were electrotransferred to polyvinylidene difluoride membranes which were then blocked overnight at 4°C with 5% non-fat dry milk in Tris-buffered saline (150 mM Tris, 20 mM Tris-HCl, pH 7.5) containing 0.3% Tween-20 (TBST). Membranes were further washed in TBST and incubated with the primary antibodies (1/1,000 dilution) in TBST containing 3% BSA, followed by a 1 hr incubation with horseradish peroxidase-conjugated anti-rabbit IgG (1/2,500 dilution) in TBST containing 5% non-fat dry milk. Immunoreactive material was visualized by enhanced chemiluminescence (Amersham Biosciences, Baie d'Urfé, QC).

Gelatin Zymography

Twenty microgram protein from normal kidney (NK) and from tumor biopsies were subjected to SDS–PAGE in the presence of 0.1 mg/ml gelatin. The gels were then incubated in 2.5% Triton X-100 and rinsed in ddH_2O. The gels were further incubated at 37°C for 20 hr in 20 mM NaCl, 5 mM $CaCl_2$, 0.02% Brij-35, 50 mM Tris-HCl buffer, pH 7.6, then stained with 0.1% Coomassie Brilliant

blue R-250 and destained in 10% acetic acid/30% methanol. Gelatinolytic activity was detected as unstained bands on a blue background.

Statistics

All data were expressed as mean ± SE. Throughout the results and figure legends, the term n = x is used to indicate the number of independent experiments (x) performed. Statistical significance was determined with an ANOVA; a weighted Bonferroni analysis (stringency adjusted to allow for multiple comparisons) was performed to determine the differences between groups within an ANOVA. **P*-values of less than 0.05 were considered significant.

RESULTS

The Protein Levels of LRP and RAP Decrease in Stages III, IV, and Anaplastic Wilms Tumors

The protein levels of LRP were assessed in samples from NKs and from WT of different stages. The protein expression of the 85-kDa β-subunits was assessed by immunoblotting. We observed that the β-subunit of LRP was significantly diminished in stage III-, IV-favourable histology, and anaplastic WT when compared to its expression in NKs (Fig. 1A). Notably, the amount of this subunit was decreased by 75% (Fig. 1B) in stage III WT that are characterized by local infiltration into vital structures. The protein expression of RAP, an intracellular LRP chaperone, was also found to be rapidly decreased (Fig. 2A) by approximately 50% at stage II WT (Fig. 2B). The relevance of changes in LRP and RAP in WT was further confirmed when no significant changes was observed in the house keeping gene β-actin protein expression within each of the WT stages and throughout the samples tested (not shown). These observations suggest that RAP-mediated transport of LRP is down-regulated during the acquisition of the invasive character of WT in advanced stage disease. More importantly, this also suggests that crucial LRP-mediated functions, such as ECM protease recycling, may ultimately be significantly altered in WT.

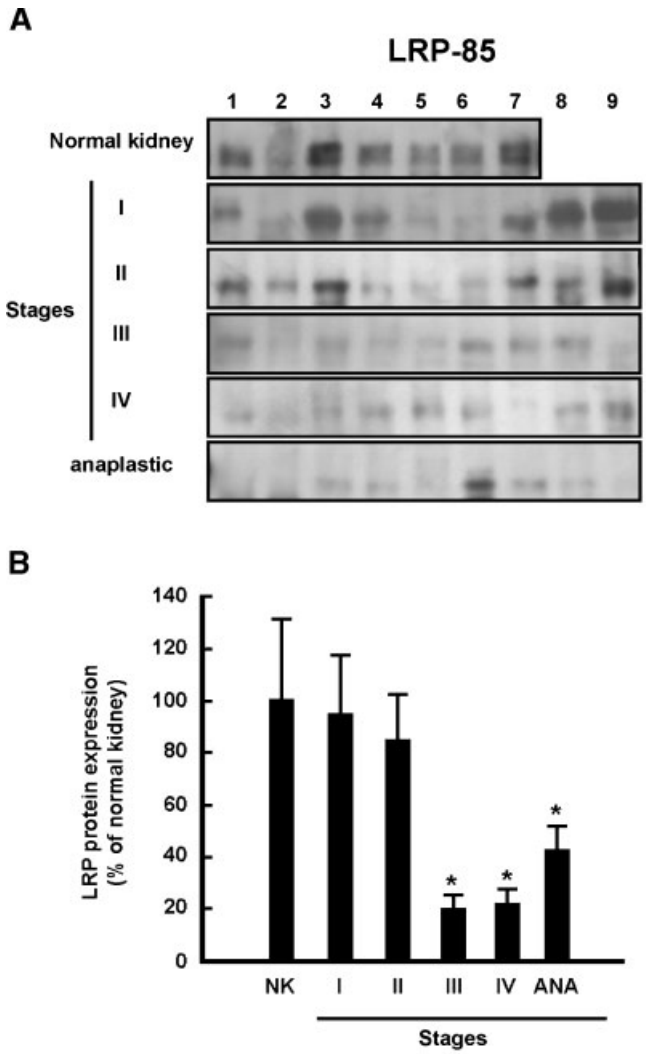

Fig. 1. LRP protein expression decreases during the acquisition of an invasive phenotype in WT. Tissue biopsies were homogenized and separated by SDS-PAGE (25 μg protein/well). **A**: Immunodetection of the 85-kDa β-subunit of LRP was performed as described in the Materials section. **B**: Quantification of LRP-85 protein levels was performed with densitometry and is expressed as percent of values from normal kidney (NK) biopsies. Statistical significance was determined with an ANOVA. **P*-values of less than 0.05 were considered significant.

ProMMP-9 Hydrolytic Activity Correlates With the Acquisition of the Invasive Phenotype of Wilms Tumors

One crucial protease associated with tumor progression which LRP internalizes for degradation is the 92-kDa MMP-9 [27]. We thus assessed the MMP-9 hydrolytic activity using gelatin-zymography, an assay that allows discriminating between the latent (pro) and active forms of MMP. We found that latent proMMP-9 gelatinolytic activity was significantly increased by about twofold in stages III, IV, and in anaplastic WT, when compared to NK (Fig. 3A and B). Evidence for plasminogen activator inhibitor-1 (PAI-1) induction and decrease in urokinase plasminogen activator (uPA) respective protein expression at stage IV and anaplastic WT (not shown) suggests potential low proMMP-9 activation subsequent to diminished plasminogen to plasmin conversion [28], and that proMMP-9 contributes very little to the ECM protein hydrolysis required for the invasive character of WT.

ProMMP-2 Activation and MT1-MMP Proteolytic Processing Increases During Wilms Tumors Progression

The other gelatinase that may also contribute to the invasive phenotype of WT is MMP-2. This gelatinase is

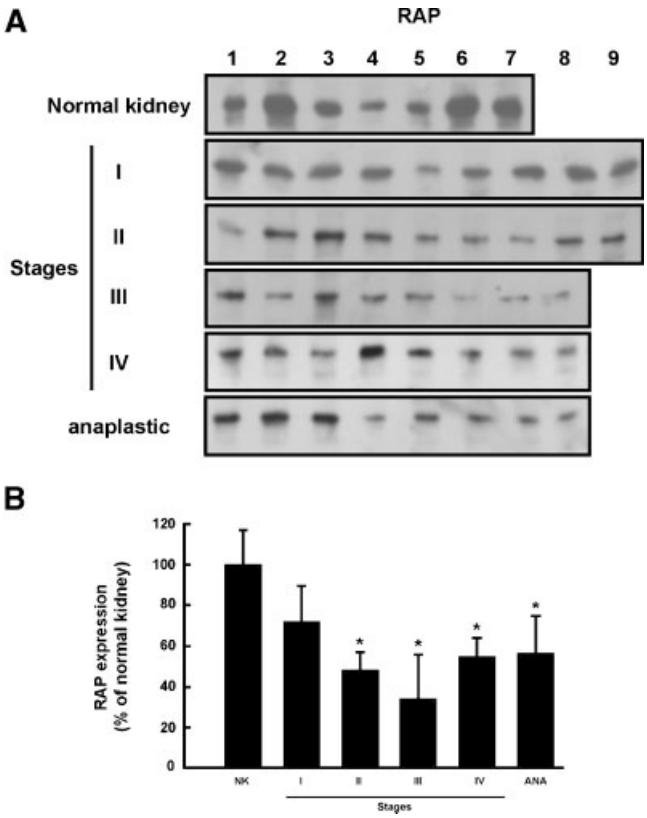

Fig. 2. RAP protein expression rapidly decreases during WT development. Tissue biopsies were homogenized and separated by SDS–PAGE (25 μg protein/well). **A**: Immunodetection of RAP was performed as described in the Materials section. **B**: Quantification of RAP protein levels was performed with densitometry and is expressed as percent of values from NK biopsies. Statistical significance was determined with an ANOVA. **P*-values of less than 0.05 were considered significant.

also known to be recycled through an LRP-mediated mechanism when complexed to thrombospondin-2 [29]. Most importantly, the latent proMMP-2 form is activated by a membrane-bound MT1-MMP whose expression correlates with several pathological disease progressions [30]. Interestingly, levels of the latent proMMP-2 form were not significantly altered regardless of the stage of the WT tested. However, we observed an increase in the activation of proMMP-2 by about threefold in stage II-IV tumors and anaplastic WT (Fig. 3A and B). Since such activation is thought to be mediated in part by MT1-MMP, we decided to assess the expression and activation status of MT1-MMP (Fig. 4A). We have previously demonstrated that the proteolytic activation of MT1-MMP leads to the formation of a 43-kDa membrane-bound inactive form that is closely associated with the regulation of ECM proteolysis in glioma cells [31,32]. The association of increased immunoreactive MT1-MMP fragment of 43-kDa (Fig. 4A) in advanced stage WT thus reflects the dynamic action of MT1-MMP which is proteolytically cleaved during proMMP-2 activation [33]. We observed that MT1-MMP proteolytic processing to its 43-kDa form was lacking in NK as well as in stage I WT specimens. Rather, an intermediate immunoreactive form was observed in the NK samples which disappeared in the WT specimens. The inactive 43-kDa form was however significantly increased in stages II to IV, as shown by the ratio of inactive 43-kDa / active 60-kDa proteins (Fig. 4B).

Multifactorial Analysis Correlates MT1-MMP Proteolytic Processing to a Potential Regulation of LRP Functions

The observed changes in the ratios of different MT1-MMP forms were further analyzed to determine their significance and impact on WT progression. We performed multifactorial analysis in order to correlate MT1-MMP proteolytic processing to either LRP expression or proMMP-2 activation. Moreover, LRP expression was also analyzed with respect to MMP-2 activation status. Analysis reveals that a clear inverse correlation exists between LRP expression and MT1-MMP proteolytic status (Fig. 5A). We have intentionally circled the ''exceptions'' (representing the intra-group heterogeneity) that deviate from our working hypothesis. Rather than cause and effect, this may suggest that alternate pathways are seen in some tumors to account for their invasive behavior. The impact of such association is further demonstrated by the inverse correlation between LRP and its documented function in regulating MMP-2 (Fig. 5B). The less LRP expression we observe the more active MMP-2 levels is found. This supports the impact that MT1-MMP may exert on LRP functions in recycling MMPs. Intriguingly, no correlation was possible between MT1-MMP and MMP-2 respective activation status within the pooled WT specimens (Fig. 5C). This suggests that alternate pathways may account for the activation of proMMP-2 in WT, and this may be a yet unexplained consequence of the biphasic tendency observed in the MT1-MMP proteolytic status in WT (Fig. 4B).

TIMP-1 is Specifically Increased in Stage III, IV, and Anaplastic Wilms Tumors

TIMPs play pivotal roles in the regulation of ECM metabolism. Their most widely recognized function is as inhibitors of MMPs. We have used Western blotting to assess TIMP-1, -2, and -4 protein levels in different stages of WT. While TIMP-2 protein levels remained unchanged (Fig. 6A and B), TIMP-4 levels were decreased by about 30% in stage II–IV tumors and in anaplastic WT, as was previously reported [34] (Fig. 6A and B). In contrast, the protein expression of TIMP-1 significantly increased when compared to NK samples (Fig. 6A). The TIMP-1 induction was observed in stage III tumors (1.7-fold), reached a peak in stage IV tumors (2.9-fold), and remained

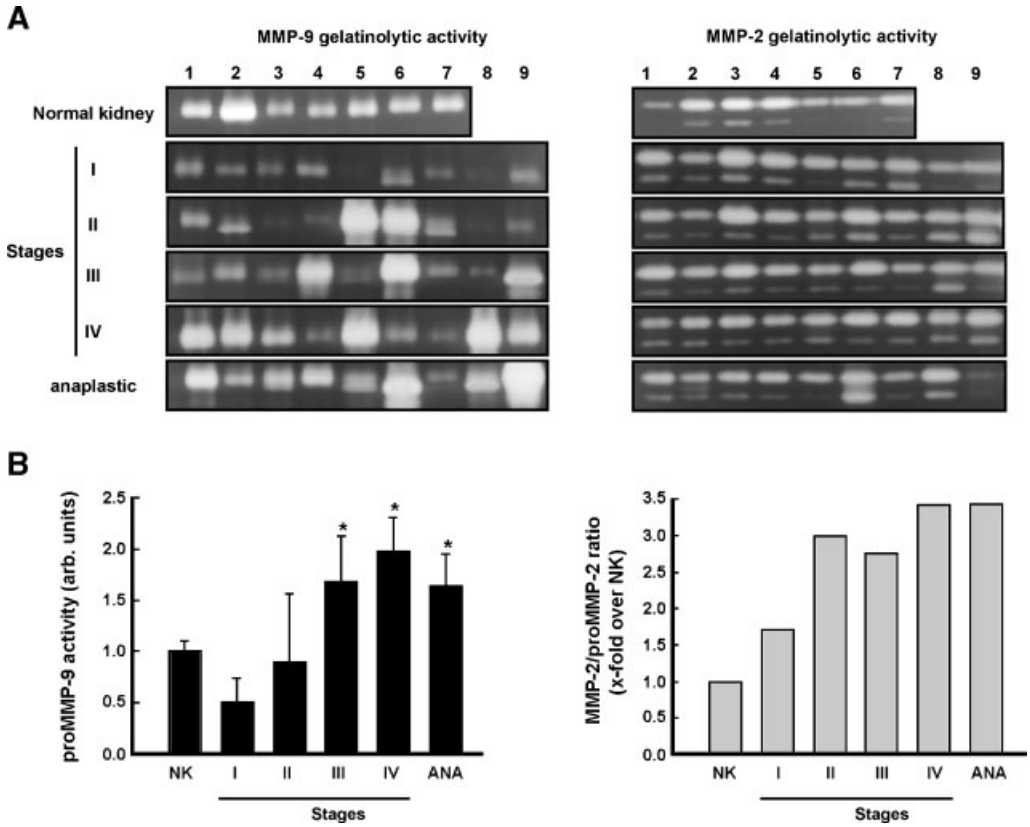

Fig. 3. ProMMP-9 gelatinolytic activity and proMMP-2 activation increase during stage progression of WT. Tissue biopsies were homogenized and zymography performed by SDS–PAGE (30 μg protein/well) in gels containing gelatin as described in the Methods section. **A**: proMMP-9 gelatinolytic activity was observed at approximately 92 kDa, while that of proMMP2 was seen at 72 kDa and that of MMP-2 detected at 62 kDa. **B**: Quantification of the extent of gelatin hydrolysis was performed by densitometry for both MMP-9 (black bars) and MMP-2 (gray bars) gels. The proMMP-2 activation status was expressed as the active MMP-2/latent proMMP-2 gelatinolytic bands for each stage. Statistical significance was determined with an ANOVA. **P*-values of less than 0.05 were considered significant.

significantly increased in the anaplastic WT (2.1-fold) as compared with NK biopsies (Fig. 6B).

DISCUSSION

The staging system used to describe WT progression considers several physical features of the tumor that increase the risk of local or distant recurrence and therefore dictate therapeutic modifications [1]. However, new molecular markers are needed since morphological evidence as well as the focal or diffuse nature of anaplasia is, for instance, thought to be a marker of chemoresistance of the tumor rather than evidence of aggressiveness or tendency to disseminate. To date, the most documented molecular marker is the zinc finger protein WT1; which loss-of-function mutations in the *WT1* gene, however, are only responsible for ∼15% of sporadic WT, suggesting a more complex involvement of additional molecular players in tumorigenesis and ECM remodelling than had been predicted. In the present study, we provide evidence for a new molecular mechanism that is defective in the recycling of proteins involved in ECM degradation and for which chronic accumulation correlates with the invasive phenotype of WT. Although considered as a ''favourable histology,'' we suggest that stages I to IV be potentially subdivided into non-invasive (I–II) and invasive (III–IV) tumors according to molecular processes such as a downregulation of LRP level and activation of MT1-MMP that could account for the invasive and metastatic phenotype seen in some WT.

High rates of ECM turnover characterize both kidney development and renal diseases, indicating that similar mechanisms are at work regulating the balance between MMPs and TIMPs [38]. In our study, we observed a significant decrease in TIMP-4 expression, as had previously been reported for WT [34]. Interestingly, one role for TIMP-4 was recently described for binding and inhibiting MT1-MMP autocatalytic processing [39]. The decrease in TIMP-4 may thus partly explain the increase in MT1-MMP proteolytic processing that we observed in WT stage progression. Since a decrease in the amount of TIMPs, relative to MMPs, could promote tumor progression, we also analyzed TIMP-1 and TIMP-2 expression since they possess high inhibitory capacity for MMP-2 and

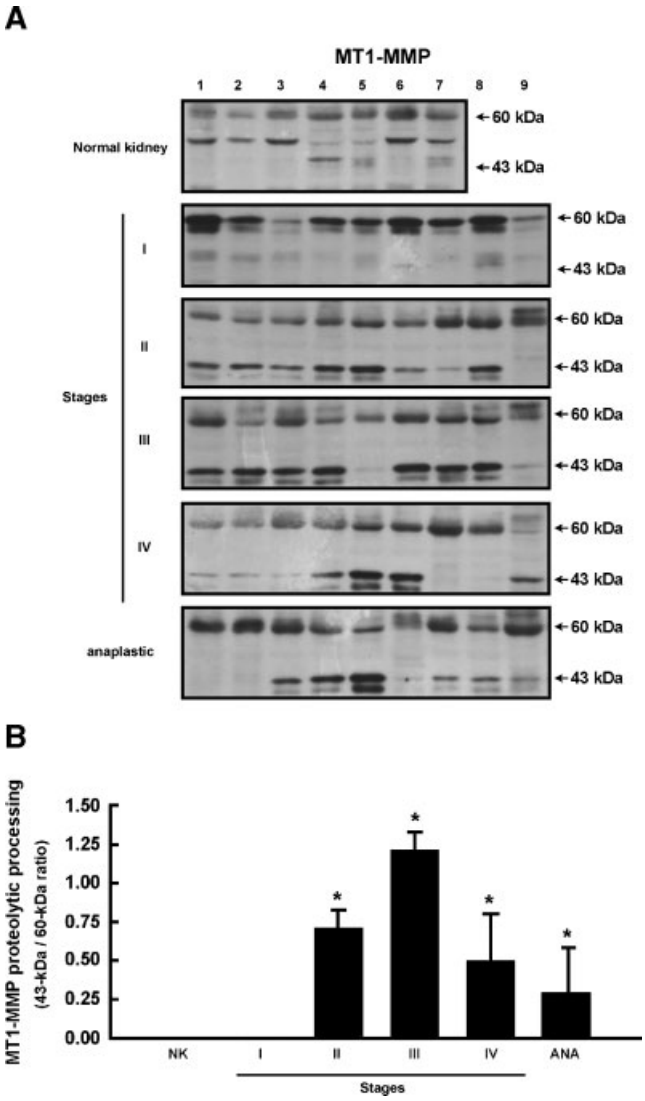

Fig. 4. MT1-MMP proteolytic processing is increased in stages II–IV, and anaplastic WT. Tissue biopsies were homogenized and separated by SDS–PAGE (25 μg protein/well). **A**: Immunodetection of the active (60-kDa), intermediate (52-kDa), and inactive (43-kDa) MT1-MMP immunoreactive forms was performed as described in the Materials section. **B**: Quantification of MT1-MMP active and inactive protein levels was performed with densitometry and is expressed as the ratio of inactive over active MT1-MMP forms for NK and for samples from WT at different stages. Statistical significance was determined with an ANOVA. **P*-values of less than 0.05 were considered significant.

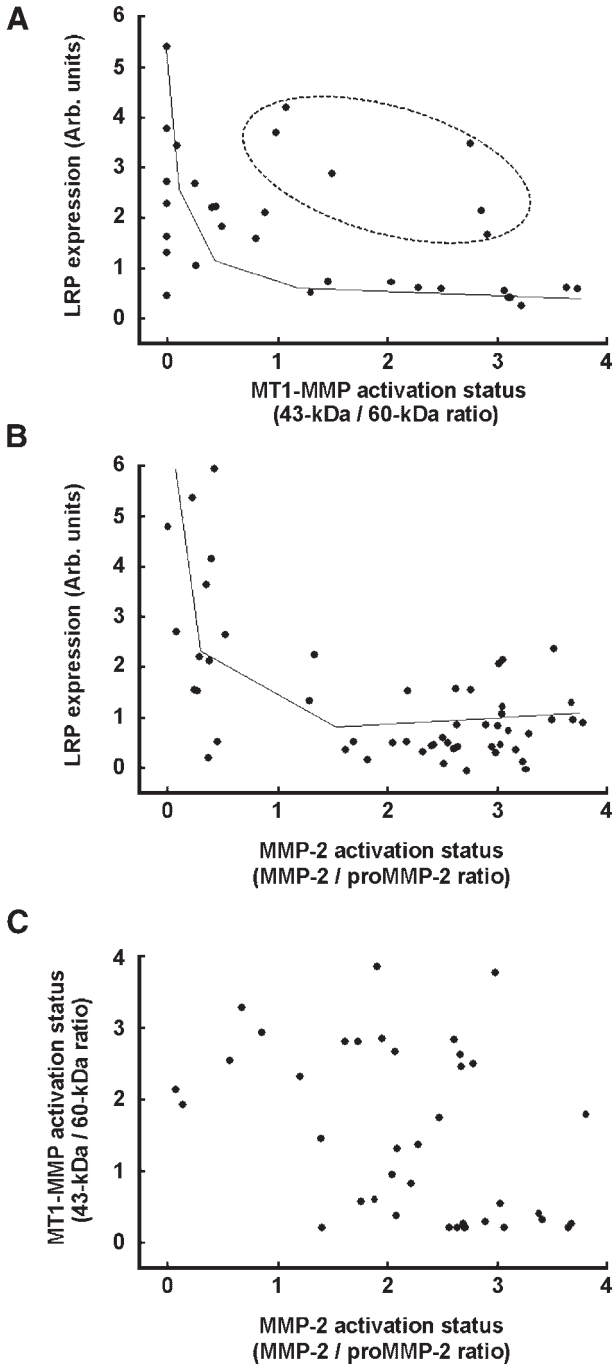

Fig. 5. MT1-MMP proteolytic processing inversely correlates with LRP expression but not with proMMP-2 activation. **A**: Multifactorial analysis was performed in order to correlate the MT1-MMP activation status to that of LRP (A). Activated MMP-2 levels were also correlated to LRP expression (**B**) and MT1-MMP activation status (**C**). A total of 52 samples from all the WT stages and NK were plotted. Exceptions that deviate from our working hypothesis are circled in (A) and reflect potential intra-group heterogeneity.

MMP-9, whose levels of protein expression are crucial in the growth of many tumor types. Surprisingly, while TIMP-2 expression remained unaltered, TIMP-1 expression was highly increased. This result prompted us to explore whether any of the soluble MMP-2 or MMP-9 activity was also altered. We found that proMMP-9 expression was significantly increased during WT stage progression when compared to NK specimens.

Changes in the activity and protein expression patterns of MMP-2, MMP-9, TIMP-1, and TIMP-2 were further explored and correlated to the expression of LRP which, among other roles, is known to be involved in the recycling of extracellular complexes of TIMP-1:MMP-9 and uPA:PAI-1 [40]. The expression of LRP, as well as that

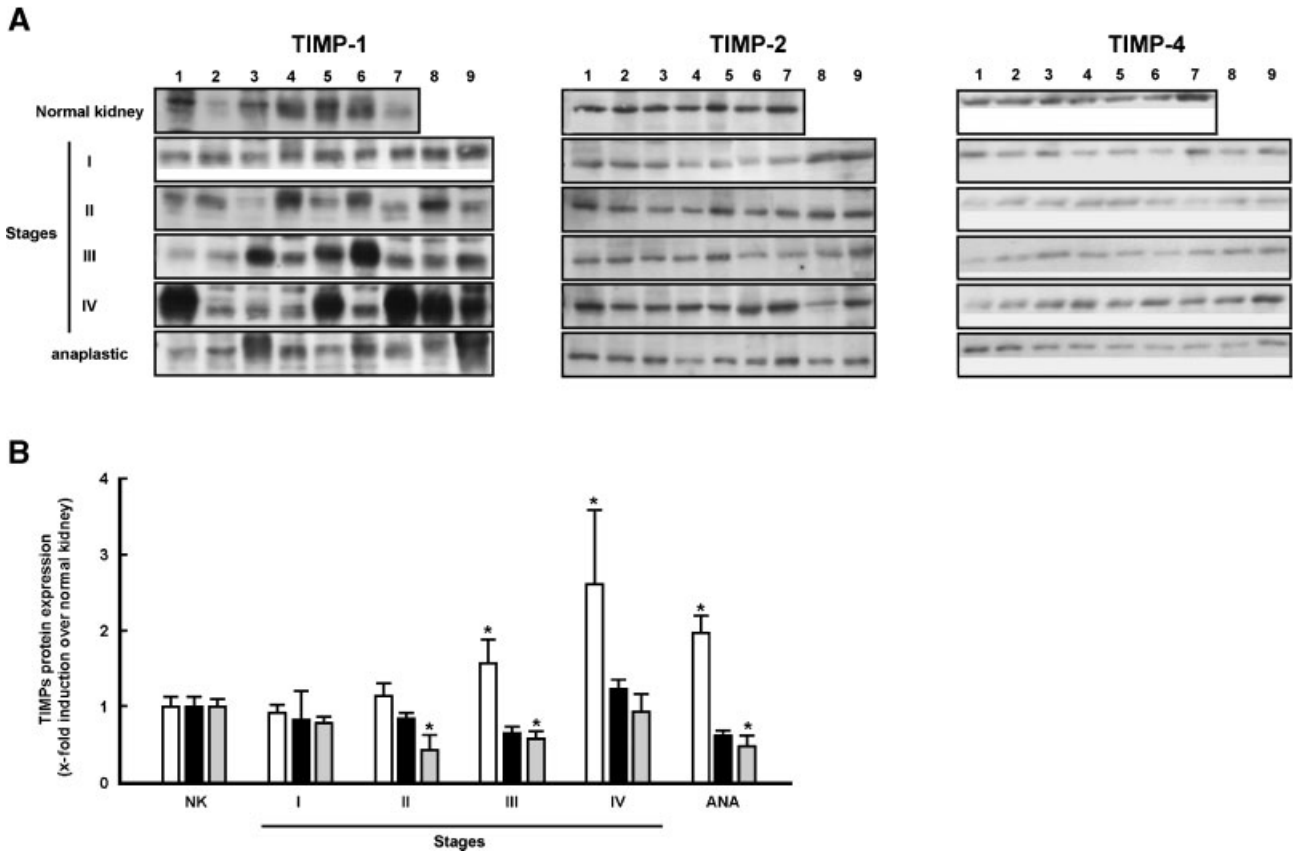

Fig. 6. TIMP-1, but not TIMP-2 or -4, protein levels increase in stages III–IV, and anaplastic of WT. Tissue biopsies were homogenized and separated by SDS–PAGE (25 μg protein/well). **A**: Immunodetection of TIMP-1, -2, and -4 was performed as described in the Materials section. **B**: Quantification of TIMP-1 (open bars), TIMP-2 (black bars), and TIMP-4 (gray bars) protein levels was performed with densitometry and is expressed as fold induction over NK biopsies. Statistical significance was determined with an ANOVA. * *P*-values of less than 0.05 were considered significant.

of RAP, an intracellular chaperone which binds tightly to LRP, is decreased in tumors of stage III–IV and anaplastic WT. Whether these decreases could be attributable to downregulation in gene expression remains to be established. However, the α-subunit of LRP was recently shown to be a substrate for proteolytic regulation by the cell surface MT1-MMP in cells stably transfected with the MT1-MMP gene [23]. In fact, both LRP and RAP were cleaved by MT1-MMP in vitro [23]. To date, it appears that the only MT1-MMP-mediated cleavage sites of LRP are localized in the C-terminal, membrane-adjacent portion of the α-subunit of LRP. Further studies will be needed to establish whether the membrane-anchored β-subunit is also susceptible to cell surface proteolysis. As this study shows that MT1-MMP activation status is significantly upregulated in advanced stage WT, as reflected by the extent of MT1-MMP proteolytic processing, it is tempting to suggest that such a dynamic function of MT1-MMP would regulate LRP and ultimately contribute to WT development. MT1-MMP-mediated activation of the latent proMMP-2 into its active MMP-2 form was however ruled out because of no significant correlation between these two parameters in intra-sample correlation analysis of our WT specimens. Collectively, our data point to a potentially central role for MT1-MMP, whose multiple actions may at least downregulate LRP and RAP functions, which together result in an accumulation of LRP ligands in the ECM environment leading to increased ECM degradation. These data further underscore a potential LRP functional defect that correlates with an invasive phenotype in WT. The major steps contributing to of this phenomenon are summarized in the scheme of Figure 7. The confirmation of the acquisition of an infiltrative phenotype in WT would eventually be immensely strengthened when functional assays are performed. These may include, for instance, manipulation of expression or activity blocking of MT1-MMP with anti-MT1-MMP inhibitors such as Ilomastat or EGCg, and study the impact on one of the other proteins (such as LRP, RAP) in WT cell lines.

Among the intracellular regulation agents which could be responsible for WT development are the transcription factors that regulate gene expression for several of the proteins involved in degradation of the ECM. Among

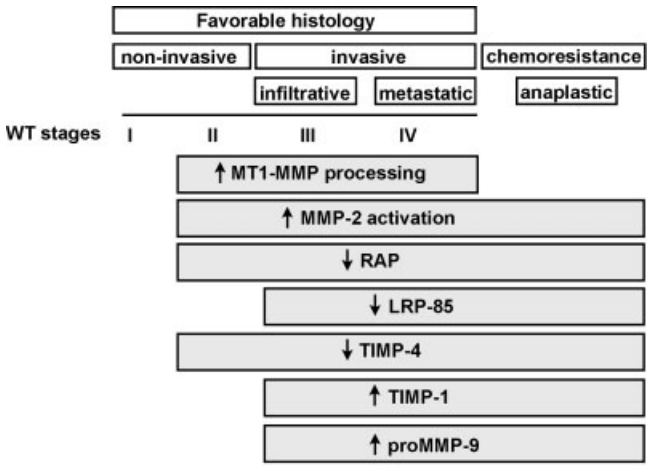

Fig. 7. Regulation of ECM degrading proteins involved in the setting of the invasive phenotype of WT. This scheme summarizes our working model in the setting of WT invasive phenotype as observed by the respective levels of key proteins regulating ECM remodelling in WT development. These proteins generally relate to the potential ligands that LRP regulates in the ECM environment. Note that MT1-MMP, whose proteolytic processing did not correlate with the activation of proMMP-2, may however play a dynamic function in regulating LRP cell surface expression and function. Although stages I to IV are qualified as ''favorable histology,'' we suggest that the WT invasive phenotype is initiated at stage II and is well established at the molecular level at stages III and IV. These latter stages are characterized by their infiltrating and metastatic phenotype, while the anaplastic stage is rather characterized by its chemoresistant phenotype.

these factors, the WT1 and Egr-1 transcription factors are highly homologous in their zinc-finger domains, but they regulate transcription from reporter constructs differently [41] and have opposite effects on cell growth [42]. It is thus possible that the opposing effects of WT1 and Egr-1 on tumor growth are due to opposite regulation of common target genes. Indeed, binding of the WT1 zinc finger protein to the Egr-1 consensus sequence [8,43] may potentially regulate the expression of genes that possess such a common DNA binding site, as does MT1-MMP [44]. Recently, an overlapping Sp1/Egr-1 binding site was identified to regulate *MT1-MMP* transcription in glomerular mesangial cells [45]. This report suggests that transcriptional regulation tightly controls the tumorigenic process in kidney, and may control the stage of WT. Interestingly, Egr-1 was recently suggested to abrogate the effects of WT1 on growth regulation [46]. Indeed, elevated levels of Egr-1 have been reported to enhance WT growth [46] possibly through MT1-MMP functional upregulation as suggested by our data on WT biopsies. Aberrant expression of Egr-1 may thus significantly contribute to continuous growth instead of differentiation and, therefore, play an important role in the setting of WT.

An Sp1 binding site was also identified in the *WT1* promoter, and abundant Sp1 is thought to be a prerequisite for *WT1* gene regulation and expression [47]. The expression pattern of Sp1 during nephrogenesis suggests further regulation of other genes involved in ECM degradation and tumor progression which possess Sp1 binding sites such as in the genes for LRP [48], MMP-9 [49], and MT1-MMP [50]. Interestingly, phosphorylation of the transcription factor Sp1 was reported to increase its binding affinity for its site on the MT1-MMP promoter in microvascular endothelial cells, and to reduce the ability of Egr-1 to displace it, leading to decreased MT1-MMP expression [51]. Further studies will be needed in order to delineate the complex crosstalk and respective roles of these transcription factors in the different stages of WT.

In summary, we have identified and correlated the expression and potential functional regulation of LRP during WT development to increased MT1-MMP-mediated processes. Interestingly, sequence-specific silencing of MT1-MMP expression was recently shown to suppress tumor cell migration and invasion [52]. This further highlights the importance of MT1-MMP as a potential therapeutic target for invasive tumors [53,54]. Our data suggest that the etiology of WT may then, in part, be related to the defective recycling of ECM-associated proteolytic enzymes. This mechanism may explain the increased invasive potential of the developing tumor, but will have to be extended to matched pairs of normal fetal kidneys. The elucidation of the mechanisms involved in WT progression will help design new therapeutic modalities targeting this pediatric cancer.

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