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The Promyelocytic Leukemia Protein Is Upregulated in Conditions of Obesity and Liver Steatosis

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The promyelocytic leukemia protein (PML) is the essential component of multi-protein sub-nuclear structures, the PML-Nuclear bodies (PML-NB, [1]). The PML-NBs regulate a variety of nuclear functions, including post-translational modifications in nuclear proteins (acetylation, SUMOylation, ubiquitylation) [1]. This function has led to the current notion that PML is a modulator of cell responses, which might not be essential in steady-state conditions (under lack of stress) but plays a critical role when the cell and organism is challenged. This notion is supported by the fact that the lack of PML in mice does not result in embryonic lethality or overt physiological alterations in adulthood, but it alters the response to oncogenic insults or dietary alterations [1-4].

The activity of PML has been mostly investigated in the context of suppression, pathogenesis and progression of tumorigenesis. However, evidence of the function of this protein beyond malignant transformation are quickly accumulating. We and others have recently reported that the expression of PML is relevant for the response to metabolic insults, nutritional disorders and obesity [4-8]. In this study, we aimed at extending and defining the status of PML in conditions of nutritional challenge. To this end, we evaluated PML transcript abundance in a cohort of human liver biopsies from lean or morbidly obese subjects. Liver material from lean patients was obtained from 3 subjects (3 women; age, 46±12 years; BMI, 21±2 kg/m²) undergoing partial hepatectomy for benign tumors (neighbor tissues from four adenoma and one focal nodular hyperplasia) and did not display any hepatic steatosis, inflammation or fibrosis. For obese patients, bariatric surgery was indicated in accordance with French guidelines (the study was approved by “Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Nice” 07/04:2003, N° 03.017) (Fig. 1A). Quantitative real time RT-PCR (q-RT-PCR) analysis (references from Applied biosystems Hs99999902_m1 for housekeeping RPLP0 (Ribosomal protein, large subunit, P0) and Hs00231241_m1 for PML and as previously described [9, 10]), revealed a significant PML up-regulation in obese patients without liver complications (n=5) versus lean subjects (+2.49±0.28, P=0.036). We next decided to extend this observation to diet-induced (Research Diets D12451 as obesogenic
diet; LabDiet 5008 (Pharmaserv, Framingham, MA, USA) as chow, following the procedure in Ref. [11]) or genetic (Agouti mutant heterozygous) mouse models of obesity (the mouse experiments were approved by IACUC, USA). Q-RT-PCR analysis in high-fat diet fed mice demonstrated the up-regulation of Pml in livers from obese mice (murine Pml primers, Pml_F: GATCTCCGACAAATTCAGT, Pml_R: ATGCCACCT GCTGAATCTCCT; Cyclophilin was used as housekeeping gene; Cyclo_F: GGTGGAGAGCACCAAG ACAGA, Cyclo_R: GCCGGAGTCGACAATGATG; Fig. 1C). Importantly, transcriptional up-regulation of this gene was accompanied by the detection of Pml immunoreactive nuclear bodies by immunohistochemistry in <5% of hepatocytes in livers from HFD mice (using Millipore Pml antibody), but rarely in those from lean individuals (Fig. 1D), similar to our prior observations in another mouse model [4]. Of note, Pml immunoreactivity was often restricted to a single or few large nuclear bodies of doughnut shape, a structural conformation prior described for PML nuclear bodies. We also confirmed the increase Pml immunoreactivity in a second murine model of obesity, driven by the heterozygous mutation in the Agouti gene (Jackson laboratory ref. KK.Cg-Ay/J; Ref. [12]; Fig. 1E).

**Table 1.**

| Parameter                  | Liver       |
|----------------------------|-------------|
| n (F/M)                    | 23 (21/2)   |
| Age (years)                | 38 ± 2      |
| BMI (kg/m²)                | 43 ± 1      |
| T2D (n)                    | 4           |
| Fasting insulin (mIU/L)    | 14.2 ± 2.6  |
| Fasting glucose (mmol/L)   | 6.2 ± 0.6   |
| HOMA-IR                    | 4.7 ± 1.4   |
| Hba1c (%)                  | 6.2 ± 0.4   |
| Triglycerides (mmol/L)     | 1.4 ± 0.1   |
| HDL cholesterol (mmol/L)   | 1.4 ± 0.1   |
| LDL cholesterol (mmol/L)   | 3.1 ± 0.1   |
| Without NAFLD (n)          | 5           |
| With Steatosis (n)         | 18          |
| S1 (n)                     | 6           |
| S2 (n)                     | 4           |
| S3 (n)                     | 8           |
| ALT (IU/L)                 | 29.3 ± 5.0  |

Figure 1. **PML is up-regulated in hepatocytes from obese individuals.** (A-B) Description of the clinical features of obese subjects (A) in which PML transcript abundance (q-RT-PCR) was measured (B). (C-D). Pml transcript abundance (q-RT-PCR) (C) and immunoreactivity (D) in mice subjected to standard (chow) or obesity-inducing high-fat diets (HFD) (representative micrographs from n=4-5; squares represent zoomed region; red arrows depict PML immunoreactive hepatocytes; red asterisks depict PML immunoreactive non-hepatocyte cells). **, p<0.01 calculated by non-parametric Mann-Whitney test. (E) Pml, Sumo-1 and Nucleophosmin (Npm) immunoreactivity in livers from wild type or agouti heterozygous mutant mice (representative micrographs from n=3; squares, arrows and asterisks as described in D).
PML nuclear bodies are enriched in SUMO-1 (Santa Cruz BioTechnology). Immunohistochemical analysis confirmed similar nuclear distribution of Sumo-1 into nuclear bodies at a similar rate of Pml, which strongly suggests that the staining of Pml corresponds to functional nuclear bodies (Fig. 1E). It is worth noting that this altered pattern of nuclear localization was not observed in other non PML-NB resident proteins such as nucleophosmin (a nucleolar protein; Cell Signaling Technology; Fig. 1E). In summary, our results demonstrate that PML accumulates in hepatocytes in conditions of obesity.

Obesity is associated to lipid accumulation in hepatocytes, termed steatosis [13]. Since there are pathological conditions where steatosis can be triggered in the absence of obesity, we sought to ascertain whether PML accumulation was related to obesity or it was a consequence of the steatosis associated to weight increase. To this end, we first evaluated PML expression in obese patients with different degree of steatosis (0, <5%; 1, 5%-30%; 2, >30%-60%; 3, >60%) [14]. The results clearly demonstrated that PML expression significantly correlated with the extent of steatosis (Fig. 2A).

In addition, we took advantage of a dietary regime that results in weight loss and liver lipid accumulation, namely ketogenic diet (KD; Ref. Bio-Serv F3666, [11]). The results confirmed that Pml transcript and immunoreactivity were increased (Fig. 2B-C).

Fatty liver disease is a rapidly increasing pathology that, albeit tightly associated with obesity, is also observed in a fraction of lean individuals, where it is thought to be related to nutrition and lifestyle factors [13]. Our results conclusively demonstrate that PML expression is increased in livers from obese individuals, and is particularly associated to a steatotic phenotype. These findings are of relevance for the characterization of PML non-tumoral functions. Importantly, this observation might open an interesting mean of regulation of PML in the context of canerous lesions, in which nutrient availability might impact on the expression of this protein and hence on the biology of the disease. In particular, they extend our knowledge regarding the patho-physiological regulation of PML in metabolism. In this respect, it is tempting to speculate that PML induction may represent a failsafe response to steatosis in view of the positive role it plays in the activation fatty acid oxidation pathways [4, 5].

**Figure 2. PML liver mRNA expression increases in steatosis.** (A) PML mRNA expression (q-RT-PCR) in individuals with defined degree of liver steatosis; the correlation of PML and steatotic grade (S0-S3) was calculated by non-parametric Spearman test. (B-C) Pml transcript expression(q-RT-PCR) (B) and immunoreactivity (C) in livers from mice subjected to chow or ketogenic diet (KD). Representative micrographs from n=4-5; squares represent zoomed region; red arrows depict PML immunoreactive hepatocytes; red asterisks depict PML immunoreactive non-hepatocyte cells ***, p<0.01 calculated by non-parametric Mann-Whitney test.

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Competing Interests
The authors declare no competing financial interest.

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