

Human Genetics 2019- Progesterone inhibition of MDM2 p90 protein in MCF-7 human breast cancer cell line is dependent on p53 levels - Moussa Alkhalaf - Kuwait University

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Abstract

We present evidence that reestablishing of P53 expression by transient transfection of P53 cDNA in these cells enhances the expression level of MDM2 p90 isoform. Chronic neutrophilic leukemia (CNL) could be a rare myeloproliferative neoplasm characterized primarily by leukocytosis, but often lacking distinct clinical, laboratory, and molecular features. Assessing the patient with an atypical myeloproliferative picture and correctly making the diagnosis of CNL can be challenging for pathologists and clinicians alike. The aims of this report are to detail the clinical case of a 59-year-old male veteran with initial presentation of hyperleukocytosis so as to demonstrate the laboratory and clinical criteria utilized to establish a diagnosis of CNL. We also briefly review the current literature on the diagnosis and treatment of CNL.

Keywords: MDM2, isoforms, p53, p90, progesterone, MCF-7, breast cancer

INTRODUCTION:

The *mdm2* gene was originally cloned as an amplified gene on a murine double-minute chromosome in the tumorigenic 3T3DM murine cell line (Fakharzadeh et al, 1991). MDM2 expression is controlled at the transcriptional level from P53-independent (P1) and P53-responsive (P2) promoters (Zauberman et al, 1995), both encoding a 90 kDa full length MDM2 (p90) protein (Brown et al, 1999). In addition, MDM2 proteins of smaller sizes have been identified (Olson et al, 1993; Perry et al, 2000; Bartel et al, 2002). These differently sized proteins arise through either proteolytic cleavage (Pochampally et al, 1998), internal translational initiation (Saucedo et al, 1999) or alternative splicing (Sigalas et al, 1996; Matsumoto, 1998). Although the biochemical functions of these small proteins have not yet been determined, the MDM2-p90 isoform binds to and inactivates P53 tumor suppressor protein suggesting that MDM2 can function as a negative feedback regulator of P53 (Momand et al, 1992; Barak et al, 1993). Lukas et al (2001) suggested that MDM2 expression is altered in invasive breast cancer and is associated with more aggressive disease. We have recently demonstrated that the progesterone-induced growth inhibition

of the MCF-7 human breast cancer cell line was associated with down-regulation of P53 endogenous levels (Alkhalaf and El-Mowafy, 2003). Because the regulation of MDM2 expression by P53 has been proposed by several authors to be the mechanism by which P53 balances its own activity (Juven et al, 1993; Midgley and Lane, 1997; Prives, 1998), we hypothesized that the decrease in P53 levels seen in MCF-7 cells treated with progesterone would affect MDM2 expression. We report here that in MCF-7 human breast cancer cells treated with progesterone, MDM2 p90 but not MDM2 p57 is down-regulated. Overexpression of P53 in MCF-7 cells stimulated the MDM2 expression and abrogated the effect of progesterone.

MATERIALS AND METHODS:

Cell lines and culture conditions:

The breast cancer cell lines MCF7, T47D, and MDA-MB231 were kindly provided by Bohdan Wasylyk (IGBMC Core Facility, Strasbourg, France). The MCF-7 cells contain functional P53 protein localized at the nucleus (Wasylyk et al, 1999) and classified as progesterone and estrogen receptor positive. The T47D cells have a mutated type of P53 which is localized in the cytoplasm (Schafer et al, 2000) and contain both estrogen and progesterone receptors. The cells were grown in RPMI1640 medium (Gibco BRL) supplemented with 5% fetal bovine serum, glutamine and gentamicin and maintained in a 5% CO₂ humidified atmosphere in a 37 °C incubator.

Western Blot Analysis:

Cells were washed twice with PBS buffer then the preheated (95°C) lysis buffer [20 mM Tris-HCl pH 7.4, 20 mM dithiothreitol (DTT), 2 mM EDTA (sodium salt), 1% (v/v) Triton X-100, 1% (v/v) NP40, 1% (w/v) sodium deoxycholate, 1 mM sodium pyrophosphate, 1 mM sodium orthovanadate (prepared in Tris buffer) and 1 mM phenylmethylsulphonyl-fluoride] was added directly to the cell monolayer. The cells were scraped and mixed with a rubber policeman, transferred to Eppendorf tubes and centrifuged at 13000 x g for 5 min. The resulting supernatant was saved and the protein was determined by the Bradford method. Extracts were boiled for 3 min in 2 x

SDS buffer. Equal amounts of protein were loaded on 10% (w/v) polyacrylamide gels according to the method of Laemmli and then electrotransferred onto nitro-cellulose membranes. The blots were incubated first with anti-MDM2 (Ab-1, clone IF2) monoclonal antibody (Oncogene Research Product, Calbiochem). The antibody is directed against the N-terminal fragment between amino acid residues 26-169 of human MDM2 (Fakharzadeh et al, 1991) then incubated with peroxidase-conjugated anti-mouse IgG (Jackson Laboratory, USA) at 1/2000 dilution. Immunoreactive bands were visualized by incubation with luminol (according to manufacturer's instructions; ECL Western blotting detection system from Amersham). The blots were stripped and hybridized with P53 (DO1) monoclonal antibodies (Oncogene Research, Cambridge, MA, USA) and processed as for the MDM2 antibodies. The TATA- Binding Protein (TBP) monoclonal antibody was used as a loading control (kindly provided by IGBMC Core Facility, Illkirch, France). Another MDM2 antibody was used, MDM2 (C-18) purchased from Santa Cruz Biotechnology (California, USA). It is an affinity purified rabbit polyclonal antibody raised against a peptide mapping within the carboxyl terminal domain of MDM2 of human origin. A prestained SDS-PAGE molecular weight protein standards (low range) is used to estimate the molecular weights of proteins ((Bio-Rad, UK).

RESULTS:

The expression of MDM2 proteins in MCF7, T47D, and MDA-MB231 human breast cancer cell lines was analyzed by Western blotting with anti-MDM2 (Ab-1) monoclonal antibody. Figure 1 shows that, with this antibody, four MDM2 isoforms were detected, the p57, p76, p80, and p90 kDa in all three cell lines. A prominent expression of MDM2 p57 protein as compared to the p90, p80 or p76 was observed in the three cell lines used in this study. In MCF-7 cells that contained functional P53 protein, the endogenous MDM2 p90 protein level was higher than these in T47D and MDA-MB 230 cell lines (both have mutated P53 proteins). In contrast, MDM2 p57 appeared to be lower in MCF-7 as compared to its levels in the cells which have mutated P53. The high level of MDM2 p57 was observed in other breast cancer cells, a normal breast cell line and in other types of cancer cell lines .

**This work is partly presented at 8th International Conference on Human Genetics and Genetic Diseases
November 25-26, 2019 Madrid, Spain, USA**