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Review

Mortalin: a potential candidate for biotechnology and biomedicine

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Summary. Mortalin is a novel member of the hsp70 family of proteins that exhibits a different staining pattern in normal and immortal cells. It was also cloned as glucose regulated protein, GRP75 and peptide-binding protein, PBP74. It has been assigned multiple functions ranging from stress response, intracellular trafficking, antigen processing, control of cell proliferation, differentiation and tumorigenesis. The present article compiles and reviews information on multiple sites and functions of mortalin. In view of its upregulation in many tumors and transcriptional inactivation function of p53, its potential use in biotechnology and biomedicine is discussed.

Key words: Mortalin, mthsp70 chaperone, p53, MKT-077, Tumor therapy

Mouse and human mortalins

Mortalin was first cloned as a novel member of the hsp70 family of proteins present in the cytoplasmic fractions of normal fibroblasts from CD1-ICR mouse (Wadhwa et al., 1993a). An antibody raised against the full protein isolated from normal fibroblasts when used for cyto-immunochemistry revealed a cytoplasmic staining of the protein in normal cells; immortal cells showed the immunofluorescence in the perinuclear region (Wadhwa et al., 1993b). Immunocloning of cDNA from immortal cells (mot-2) and their sequence comparisons with the cDNA isolated from normal mouse cells (mot-1) revealed a difference of two amino acids in the carboxy-terminus (Wadhwa et al., 1993c). Genetic identities of mot-1 and mot-2 were obtained from mouse family studies that showed segregation of two loci in two mouse generations (Kaul et al., 2000a) illustrating that mot-1 and mot-2 are allelic in mouse and were assigned to chromosome 18 (Kaul et al., 1995; Ohashi et al., 1995). Human normal and transformed cells also have differential staining of mortalin. Whereas normal cells have pancytoplasmic staining, more than 60 transformed cell lines analyzed to-date showed nonpancytoplasmic staining patterns (Fig. 1A) (Wadhwa et al., 1995; and unpublished observations). Biochemical studies have assigned the protein to subcellular localizations including mitochondria, endoplasmic reticulum, cytoplasmic vesicles and cytosol (Domanico et al., 1993; Dahlseid et al., 1994; Webster et al., 1994; Singh et al., 1997; Soltys and Gupta, 1999; Ran et al., 2000) of which mitochondria appeared to be a primary niche. Hence the protein is also called mthsp70 (Dahlseid et al., 1994; Webster et al., 1994; Bhattacharyya et al., 1995). In contrast to the mouse situation cloning of human mortalin cDNA from various human transformed cells showed identical sequences and was assigned to chromosome 5q31.1 (Kaul et. al., 1995) Apparently, there are at least two mechanisms operating for differential distributions of the mortalin protein. One is by distinct cDNAs, mot-1 and mot-2 found in mouse, and the other by yet undefined protein modifications or cellular factors found in mouse and human cells.

Functional aspects of mortalin

Mortalin is expressed in all cell types and tissues so far examined (Wadhwa et al., 1995; Kaul et al., 1997) and is expected to perform some essential functions. Some of these predicted from its mitochondrial localization and studies on its yeast homologue ssc1 include mitochondrial import and biogenesis (Krimmer et al., 2000; Geissler et al., 2001). Mortalin expression was induced by low levels of ionizing radiation (Sadekova et al., 1997), glucose deprivation (Merrick et al., 1997), calcium ionophore (Resendez et al., 1985), ozone (Wu et al., 1999), hyperthyroidism (Craig et al., 1998; Schneider and Hood, 2000), muscle activity, and mitochondrial activity and biogenesis (Ornatsky et al., 1995; Ibi et al., 1996; Takahashi et al., 1998). Many of the human transformed and tumor-derived cells were seen to have a high level of mortalin expression (Takahashi et al., 1994; Bini et al., 1997; Takano et al.,

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1997; Kaul et al., 1998; and unpublished observations). Introduction of mot-1 cDNA to NIH 3T3 cells induced cellular senescence like phenotype (Wadhwa et al., 1993c) whereas an overexpression of mot-2 cDNAs in NIH 3T3 cells resulted in their malignant transformation (Kaul et al., 1998). Similar to the mouse mot-2 cDNA, human mortalin cDNA induced malignant transformation of NIH 3T3 cells and was thus called hmot-2 (Kaul et al., 1998). Human lung fibroblasts when stably transfected with hmot-2 cDNA underwent extended population doublings *in vitro* (Kaul et al., 2000b). It was also shown that differentiation of HL-60 promyelocytic leukemia cells was accompanied by a decreased level of hmot-2/mthsp70 expression (Xu et al., 1999). An overexpression of hmot-2/mthsp70 imparted growth advantage and attenuated their differentiation (Xu et al., 1999). Other functions assigned to hmot-2/PBP74/mthsp70/GRP75 include antigen processing, *in vivo* nephrotoxicity and radioresistance (Domanico et al., 1993; Dahlseid et al., 1994; Merrick et al., 1997; Sadekova et al., 1997). hmot-2/mthsp70-p53 complexes were also detected in the mitochondria during p53-induced apoptosis (Marchenko et al., 2000) implicating its role in transcriptional-independent apoptotic signaling. Taken together, mortalin seems to play multiple functions relevant to the control of cellular proliferation and tumorigenesis.

Mechanisms of mortalin function

Studies on yeast homologue of mortalin have shown that it acts as a mitochondrial import motor in unidirectional ATP-driven import of proteins from cytoplasm into mitochondria. This function is dependent on its anchoring to the inner mitochondrial membrane and interactions with an inner mitochondrial translocase TIM44, (Krimmer et al., 2000; Geissler et al., 2001) and is essential for survival. Malignant transformation of NIH 3T3, life span extension of MRC-5 and attenuation of differentiation of HL-60 cells by overexpression of mot-2 was explained, at least in part, by its p53 inactivation function. Mot-2 and p53 were shown to interact in the cytoplasm resulting in nuclear exclusion and transcriptional inactivation of the latter (Merrick et al., 1996; Wadhwa et al., 1998, 1999). Notably, mot-2 and p53 colocalized in human transformed, but not in normal, cells (Fig. 1B). Nuclear exclusion of wild type p53 was proposed as a possible mechanism of its inactivation for some tumors (Moll et al., 1992, 1996; Takahashi and Suzuki, 1994; Takahashi et al., 1994; Moll and Schramm, 1998). An overexpression of mot-2 in NIH 3T3 cells caused an abrogation of their serum-induced nuclear translocation of p53 (Fig. 1C). Studies using deletion mutants of mot-2 and p53 demonstrated that an amino-terminus region of mot-2 binds to the carboxy-terminus region of p53 (Kaul et al., 2001; Wadhwa et al., 2002) which was earlier defined as its cytoplasmic sequestration domain (Moll et al., 1996). These studies have suggested that mot-2 may sequester p53 in the cytoplasm by directly binding to its carboxy-terminus. Significantly, abrogation of mot-p53 interactions by MKT-077 (a water-soluble delocalized

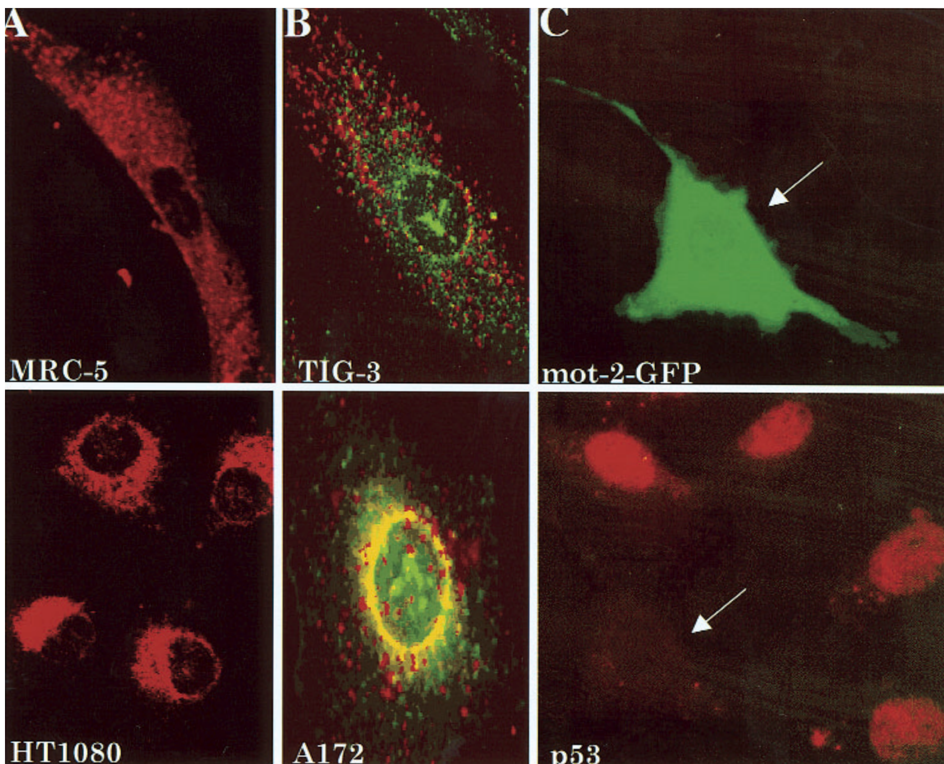

Fig. 1. A. Immunostaining of mortalin in normal (MRC-5, lung fibroblast) and transformed human (HT1080, fibrosarcoma) cells. **B.** Double immunostaining of mortalin (red) and p53 (green) in normal (TIG-3, skin fibroblast) and transformed (A172, glioblastoma) human cells (experimental and technical details described in Wadhwa et al., 1998). Colocalization of mortalin and p53 is visible as a yellow staining in A172 cells. **C.** Abrogation of nuclear translocation of p53 by mot-2. GFP-tagged mot-2 microinjected cells (visible as green fluorescence) were stained with p53 (red) after serum starvation. The mot-2 injected cells were devoid of nuclear staining for p53. Uninjected cells showed the translocation of p53 (red) to the nucleus. (experimental and technical details described in Wadhwa et al., 1998).

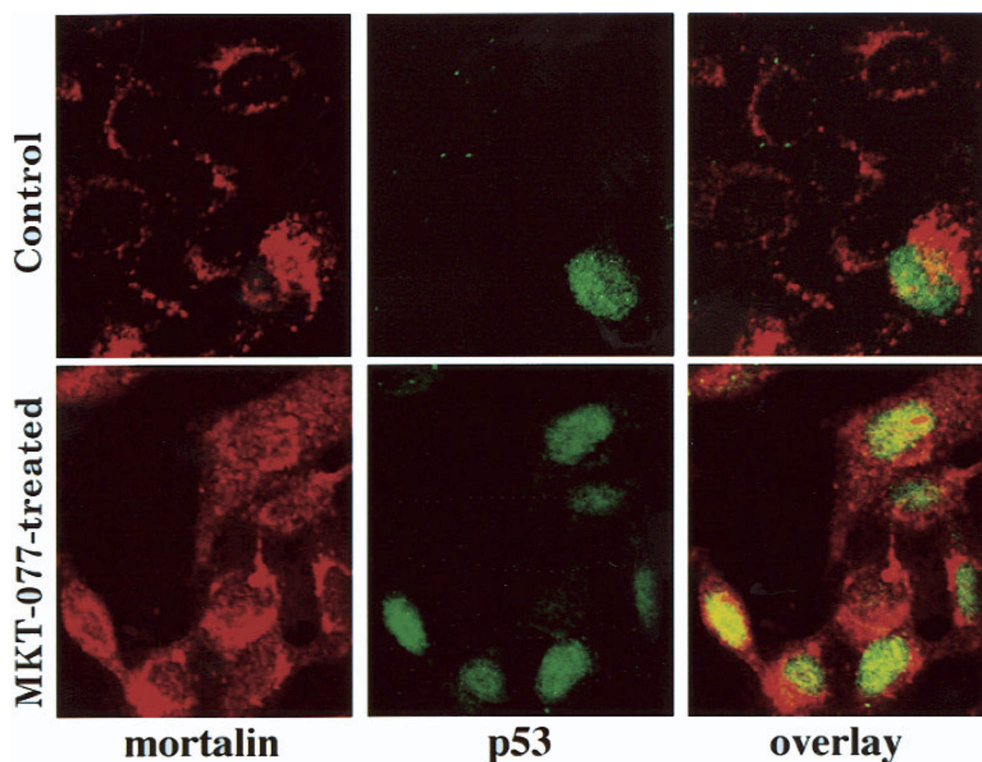

Fig. 2. Nuclear translocation of p53 in MKT-077-treated human breast carcinoma (MCF-7) cells. Cells were treated with MKT-077 and double-stained for mortalin (red) and p53 (green) (experimental and technical details described in Wadhwa et al., 2000).

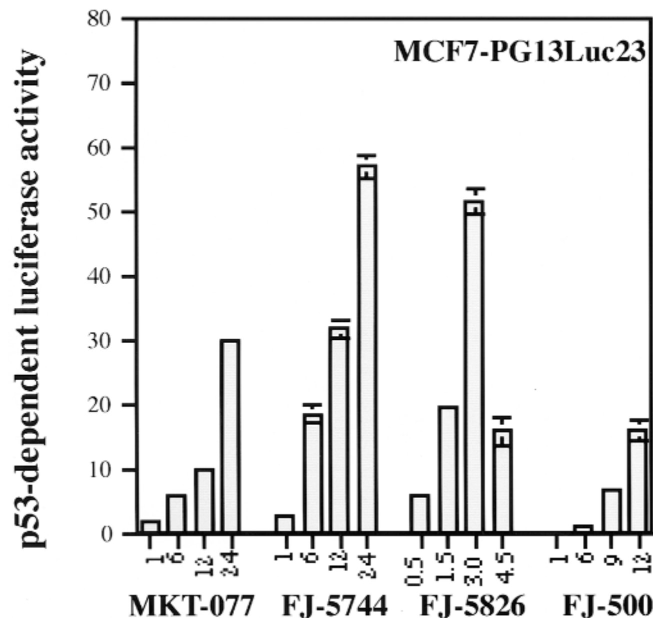

Fig. 3. p53-dependent reporter assay in MCF7 cells treated with MKT-077 and its analogues (experimental and technical details described in Wadhwa et al., 2000).

lipophilic cationic dye) that binds to mot-2 restored nuclear translocation (Fig. 2) and activation of p53 function (Fig. 3) followed by growth arrest of tumor cells (Wadhwa et al., 2000). These studies have shown that inactivation of p53 by mot-2 may be functionally significant to tumorigenesis, at least, for the tumors which retain wild type p53. Therefore, targeting of mortalin expression by antisense, antibody or ribozyme technology or abrogation of its interactions with p53 can serve as a novel approach for cancer therapeutics. Besides, inactivation of p53 by overexpression of mot-2 can be employed in *in vitro* immortalization of human cells for bioindustry and biomedicine.

Other functions of mortalin, including chaperonization and mitochondrial biogenesis, may also be significantly important for control of division potential. It was shown that it binds to FGF-1 and aids in its intracellular trafficking (Mizukoshi et al., 1999) which was mediated by its cell cycle-specific phosphorylation (Mizukoshi et al., 2001). ATP-sensitive association of mortalin with the IL-1 receptor type was also detected and predicted to have a role in receptor internalization (Sacht et al., 1999). Its binding to glucose-regulated ER chaperone, GRP94 (Takano et al., 2001), may be involved in stress resistance and survival of cells. Further studies should elucidate the significance of each of these functions in control of cell proliferation and thus tumor growth and development.

Induction of senescence in transformed cells by introduction of a single chromosome (Nakabayashi et al., 1999), chromosome-fragments and genes (Bertram et al., 1999) or chemicals (Michishita et al., 1999; Wadhwa et al., 2000) was accompanied by reversion of subcellular distribution of mortalin from non-pancytoplasmic to the pancytoplasmic type. The

mechanism of such differential localizations, role of cytoskeleton elements, downstream signal transduction and its role in regulation of proliferation warrant further studies.

Prospective

Mortalin (mot-2/mthsp70) is an essential protein belonging to the hsp70 family of chaperones. It sojourns multiple subcellular sites while residing predominantly in mitochondria, and performs multiple functions including mitochondrial import, intracellular trafficking, receptor internalization and inactivation of tumor suppressor protein p53. A differential staining pattern of mortalin may predict its different functions in normal and transformed cells and can be used for identification of cellular divisional phenotype and thus for chemical screening for tumor therapeutics. Some of its functions, such as inactivation of p53, can be employed as an advantage for immortalization of human cells *in vitro* or can be targeted for tumor therapy. Targeting of other functions such as chaperonization, mitochondrial biogenesis and intracellular trafficking may have further potentials for biotechnology and biomedicine.

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