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Pentoxifylline triggers autophagy via ER stress response that interferes with Pentoxifylline induced apoptosis in human melanoma cells

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ABSTRACT

Pentoxifylline (PTX), a non-specific phosphodiesterase inhibitor is known to inhibit the growth of various cancer cells including melanoma. Here in this study, we have found that PTX induces autophagy in human melanoma cell lines (A375 and MeWo). Induction of autophagy is associated with the increase in Atg5 expression as knockdown of Atg5 effectively inhibited PTX mediated autophagy. A decrease in mTOR activation was also observed after PTX treatment. We observed that autophagy was activated as a downstream effector mechanism of ER stress induced by PTX. ER stress response was confirmed by upregulation of IRE-1α, GRP78 and CHOP expression. PTX treatment also resulted in an increase in intracellular calcium (Ca^{2+}) level. Ca^{2+} is the central player as blocking Ca^{2+} by intracellular calcium chelator (BAPTA-AM) effectively inhibited the PTX induced ER stress response as well as autophagy. Moreover, silencing of CHOP also resulted in autophagy inhibition with a decrease in Atg5 expression. Collectively, PTX triggers ER stress response followed by induction of autophagy via involvement of Ca^{2+}→CHOP→Atg5 signalling cascade. Interestingly, inhibition of intracellular calcium level by BAPTA-AM significantly increased PTX mediated cell death by augmenting intrinsic apoptotic pathway. Inhibition of autophagy by the ATG5 siRNA and pharmacological inhibitor, chloroquine also enhances PTX induced cell death. Taken together, our results clearly indicate that activation of ER stress response and autophagy provides resistance to PTX mediated apoptosis, and thus, interferes with the anticancer activity of PTX in human melanoma cells.

1. Introduction

Melanoma is one of the lethal forms of skin cancer and the incidence rate of melanoma continues to increase rapidly [1]. Melanoma is highly resistant to apoptosis because of the constitutive activation of BRAF and NRAS signalling pathways which lead to over expression of various antiapoptotic proteins (Mcl-1, Bcl-2 and Bcl-X_L, etc.) [2]. Also, upregulation of the autophagic machinery, over expression of multidrug resistance proteins, resistance to ER stress and enhanced DNA repair mechanisms further confer resistance to drug-induced apoptosis in melanoma [3,4]. Surgical removal is the only treatment available in early stages but once the cancer has metastasised it is very difficult to treat and the five year survival rate remains less than 5% [5]. For the treatment of metastatic melanoma, two BRAF inhibitors, Vemurafenib and Dabrafenib are currently in use. These two inhibitors have shown remarkable results in limiting the growth of BRAF mutated cancer, but there are reports which suggest that many patients treated with these inhibitors have developed resistance and even disease reversal after treatment [6]. Despite the various advancements in melanoma therapy, cutaneous metastatic melanoma remains difficult to treat. Therefore, there is a need to search new alternate approaches for melanoma therapy which can target both BRAF mutated as well as BRAF wild type cells.

A methylxanthine derivative and a non-specific phosphodiesterase inhibitor, Pentoxifylline (PTX, $C_{13}H_{18}N_4O_3$, Fig. 1A) has been shown to possess anticancer properties. PTX is known to radiosensitise various tumours and also enhance antitumor activity of many chemotherapeutic agents [7,8]. PTX also exhibits antimetastatic and antiangiogenic properties against A375 melanoma cells [9]. Earlier we have shown that PTX induces apoptosis

Abbreviations: PTX, Pentoxifylline; ER stress, endoplasmic reticulum stress; CHOP, C/EBP homologous protein; GRP78, glucose regulated protein 78; IRE1-α, inositol-requiring enzyme 1alpha; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis(acetoxymethyl ester); PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species; LC3, microtubule-associated protein 1 light chain 3; HBSS, Hank's Balanced Salt Solution.

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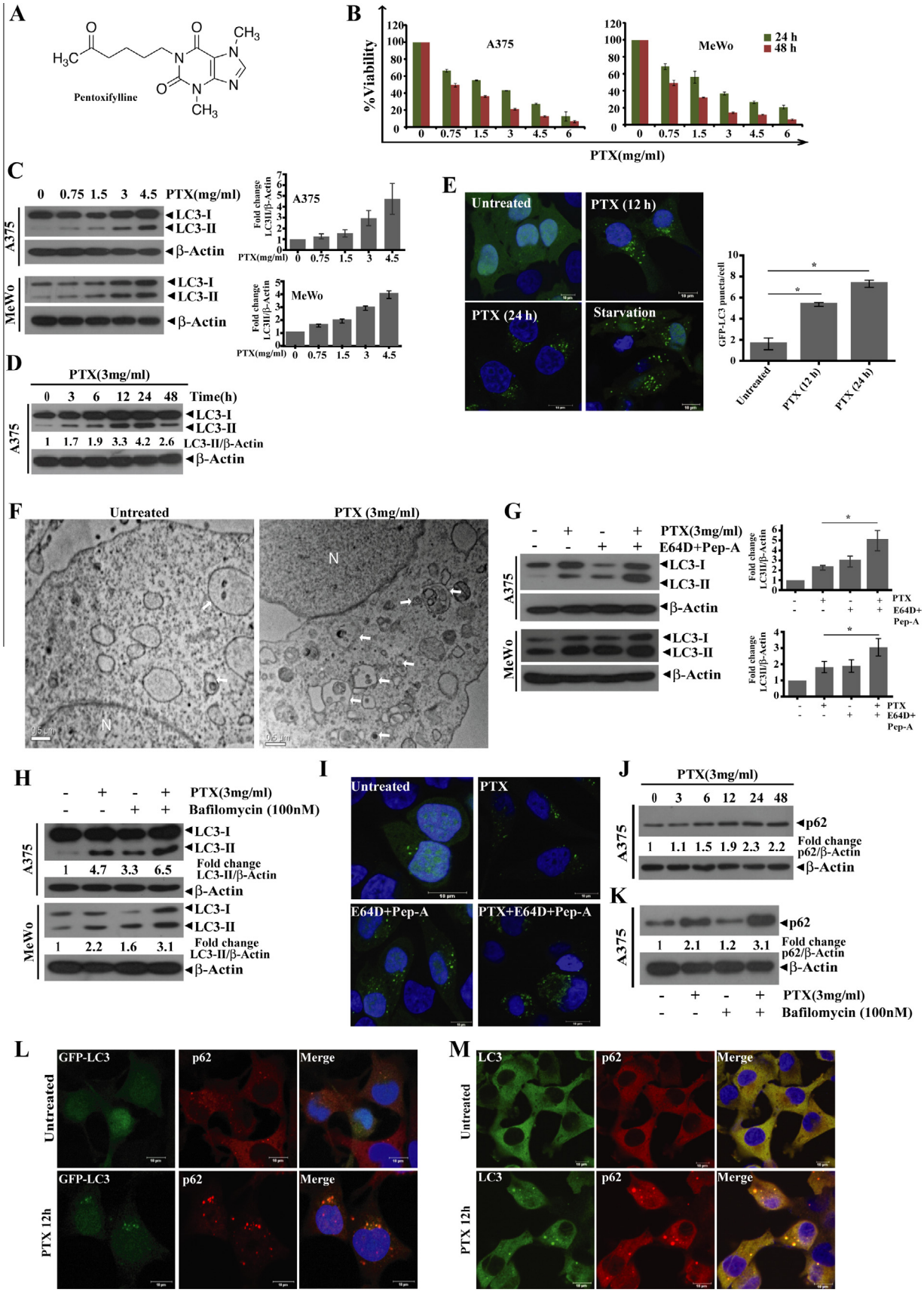
A
Pentoxifylline
B
%Viability
A375
MeWo
24 h
48 h
PTX(mg/ml)
C
PTX(mg/ml)
LC3-I
LC3-II
β-Actin
Fold change LC3II/β-Actin
D
PTX(3mg/ml)
Time(h)
LC3-II/β-Actin
E
Untreated
PTX (12 h)
PTX (24 h)
Starvation
GFP-LC3 puncta/cell
F
Untreated
PTX (3mg/ml)
N
G
PTX(3mg/ml)
E64D+Pep-A
H
Bafilomycin (100nM)
Fold change LC3-II/β-Actin
I
J
p62
Fold change p62/β-Actin
K
L
GFP-LC3
p62
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M
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