Cancer Letters 304 (2011) 60–69

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mevalonate pathway inhibitors affect anticancer drug-induced cell death and DNA damage response of human sarcoma cells

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ARTICLE INFO

Article history:
Received 24 November 2010
Received in revised form 22 December 2010
Accepted 30 December 2010

Keywords:
Mevalonate pathway
Small GTPases
Anticancer drugs
DNA damage response (DDR)
Sarcoma cells

ABSTRACT

Lovastatin (Lov), bisphosphonates (BP) and metformin (Met) are widely used drugs, having in common that they interfere with the mevalonate pathway (MP). The MP generates isoprene moieties required for the function of regulatory GTPases controlling cell proliferation and survival. Here, we addressed the question whether MP inhibitors interfere with the anti-tumor efficacy of anticancer drugs. We comparatively analyzed the effect of equitoxic doses of Lov, BP and Met on cell viability, cell cycle progression, apoptosis and DNA damage response (DDR) of human osteo- and fibrosarcoma cells exposed to doxorubicin or cisplatin. We found that Lov, BP and Met modulated the anticancer drug sensitivity of sarcoma cells in an agent-, dose and time-dependent fashion. Mostly, the MP inhibitors increased the cytotoxicity of the anticancer drugs in an additive manner. MP modulators differed from each other regarding their impact on anticancer drug-induced DNA damage response as measured by the phosphorylation status of SAPK/JNK, Chk-1 and H2AX as well as p53 protein level. In this regard, lovastatin and metformin turned out as the most effective inhibitory drugs. The data show that MP inhibitors can affect the anti-tumor efficacy of anticancer drugs and impact the DDR of human sarcoma cells.

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1. Introduction

The therapeutic efficacy of anticancer drugs, which is mainly defined by their tumor cell kill, is influenced by numerous factors. Key mechanisms determining the responsiveness of tumor cells comprise DNA repair factors [1], drug transport [2] and DNA damage-induced signaling mechanisms [3,4] regulating gene expression, cell cycle progression and, eventually, apoptosis. Fighting pre-existing and acquired mechanisms of tumor cell resistance by use of established drugs is a promising strategy to ameliorate the outcome of anticancer therapy in the short term. During the last decade, oncogenic receptor and non-receptor tyrosine kinases turned out to be excellent therapeutic targets as their inhibition largely reduced proliferation and stimulated apoptosis of malignant cells [5,6]. Notably, blocking of EGFR signaling by corresponding antibodies (i.e. trastuzumab) and small molecule inhibitors (i.e. erlotinib) [5] additionally sensitizes tumor cells to conventional (i.e. genotoxic) anticancer drugs and radiation by interfering with mechanisms of DNA repair [7,8]. Another example is the inhibition of Bcr-Abl tyrosine kinase by imatinib, which results in hypersensitivity to cisplatin by potentiation of p53-driven apoptosis [9]. These data show that targeting of tumor-specific signaling molecules efficiently improves the anti-tumor activity of conventional drugs.

Abbreviations: ATM, ataxia telangiectasia mutated; ATR, ATM and Rad3-related; Chk, checkpoint kinase; DDR, DNA damage response; ERK, extracellular regulated kinase; H2AX, histone H2AX; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; MP, mevalonate pathway; SAPK/JNK, stress-activated protein kinases/c-Jun-N-terminal kinases; XP, xeroderma pigmentosum.

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doi:10.1016/j.canlet.2010.12.022

Apart from oncogenic tyrosine kinases, Ras and Ras-homologous (Rho) GTPases play a pivotal role in the regulation of numerous cellular key processes including proliferation, tumor progression, survival and stress responses [10–14]. Ras/Rho GTPases are subject to C-terminal prenylation [15], which is required for their correct intracellular localization and physiological function. As the C15 and C20 lipid moieties originate from the mevalonate pathway (MP), it is feasible that pharmacological inhibitors of the mevalonate pathway impact the activity of regulatory GTPases and may be beneficial for tumor therapeutic purpose [16]. In line with this hypothesis, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins), which are widely used for lipid-lowering reasons nowadays, cause a down-modulation of Ras/Rho-regulated signal mechanisms. They impact genotoxic stress responses [17,18], proliferation [19] and can trigger cell death by their own [20–23]. Furthermore, they are reported to promote the cell killing efficacy of numerous anticancer drugs on various tumor cells [22,24–28]. Interestingly, as statins protect normal tissue form side effects of anthracyclines and radiotherapy [17,29–31], they might have dual beneficial functions in the therapy of malignant diseases [32].

Besides statins, bisphosphonates (BP) and metformin (Met) also interfere with the mevalonate pathway by inhibiting geranylgeranyldisphosphate and farnesyldisphosphate synthase [33] and activating AMP-kinase [34,35], respectively. As compared to statins, the impact of BP and Met on cellular responses to conventional anticancer drugs and radiation is widely unknown. Bisphosphonates are first line option in the therapy of osteoporosis [36] and other bone-related diseases, including bone metastases [37,38]. Metformin is the first line medication for the therapy of type II diabetes [39]. Since both statins, BP and Met interfere with the mevalonate pathway, although at different levels [16], we speculated that these drugs might have comparable effects on tumor cell kill and DNA damage responses triggered by anticancer therapeutics. To scrutinize this hypothesis we comparatively studied the effect of lovastatin, ibandronate and metformin on the sensitivity of human sarcoma cells (fibrosarcoma and osteosarcoma cells) to the clinically widely used and potent anticancer drugs doxorubicin and cisplatin, both of which are first-line therapy for the treatment of sarcomas. The identification of possible – beneficial or adverse – drug interactions between widely used pharmaceuticals and anticancer drugs is of particular clinical interest as it might improve or corrupt the therapeutic efficacy of anticancer therapy in cancer patients additionally suffering from widespread cardiovascular, metabolic or bone-related diseases.

2. Materials and methods

2.1. Materials

The HMG-CoA-reductase inhibitor lovastatin and FITC phalloidin were purchased from Sigma Aldrich (Taufkirchen, Germany). Metformin originates from Calbiochem (Bad Soden, Germany), Ibandronate (Bondronat®) was provided by Roche (Mannheim, Germany). Antibodies detecting ERK2 (extracellular regulated kinase 2), EGFR or Rac1 were purchased from Santa Cruz (San Diego, USA). P53 antibody was obtained from Dianova (Hamburg, Germany). p-Chk-1 antibody (p-Ser345) originates from New England Biolabs GmbH (Frankfurt, Germany). γH2AX antibody (Ser139) was from Upstate (Hamburg, Germany).

2.2. Cell culture conditions

Human fibrosarcoma cells (HT1080) were routinely grown in RPMI medium and osteosarcoma cells (U2OS) in DMEM medium (each containing 5% of fetal bovine serum) at 37 °C in humidified atmosphere.

2.3. Determination of cell viability

Cell viability was determined by use of the WST assay according to the manufacturers protocol (Roche Diagnostics, Mannheim, Germany). In order to avoid too high (i.e. human irrelevant) doses, MP inhibitors were used at a concentration where they show only moderate cytotoxicity, i.e. reduce cell viability by only about 20% (as compared to the non-treated control). If not stated otherwise, relative cell viability in untreated and MP inhibitor only treated controls was set to 100%. Correspondingly, congruent dose–response curves observed with or without MP-pretreatment and subsequent exposure to increasing doses of anticancer drugs are a sign of additive cytotoxicity. In contrast, non-congruent curves indicate that MP inhibitors either protect from or potentiate the cyotoxicity of doxorubicin or cisplatin. Data shown are the mean ± sd from up to three independent experiments each performed in triplicate. For statistical analysis, student's *t*-test was used.

2.4. Analysis of cell cycle progression and cell death

Cell cycle analysis was performed by fluorescence activated cell sorting (FACS). The SubG1 fraction was quantitated to calculate the frequency of apoptotic cells [40].

2.5. Analysis of the DNA damage response (DDR)

The protein kinases ATM, ATR and DNA-PKcs are key players in the DDR regulating cell cycle progression, repair and death [4]. Phosphorylation of the histone H2AX (γH2AX) is a commonly accepted surrogate marker of DNA damage [41]. Therefore, we investigated the level of γH2AX by Western blot analysis and, furthermore, monitored the appearance of nuclear γH2AX foci by immunohistochemistry.

2.6. Subcellular fractionation

Cells were sonicated (3 × 10 pulses; Branson Sonifier) in ice cold lysis buffer (20 mM Tris pH 7,4, 1 mM EDTA, 10 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 100 μM PMSF). Afterwards, lysate was centrifuged (10 min, 600g, 4 °C) and the precipitate (non disrupted cells) was discarded. The protein concentration of the supernatant was determined according to Bradford and the protein concentration of the samples was adjusted to 2 mg/ml. 100 μl of each

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