Brief Report

Ivermectin inactivates the kinase PAK1 and blocks the PAK1-dependent growth of human ovarian cancer and NF2 tumor cell lines

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ABSTRACT: Ivermectin is an old anti-parasitic antibiotic which selectively kills nematodes at a very low dose (0.2 mg/kg) by inhibiting their gamma-aminobutyric acid (GABA) receptor, but not mammalian counterpart. Interestingly, several years ago it was reported by a Russian group that Ivermectin can suppress almost completely the growth of human melanoma and a few other cancer xenografts in mice at the much higher doses (3-5 mg/ kg) without any adverse effect on mice. However, its anti-cancer mechanism still remained to be clarified at the molecular levels, that would determine the specific type of cancers susceptible to this drug. The first hint towards its anti-PAK1 potential was a recent finding that Ivermectin at its sublethal doses dramatically reduces the litter size (number of eggs laid) of the tiny nematode *C. elegans*. Interestingly, either a PAK1-deficiency (gene knock-out) or treatment with natural anti-PAK1 products such as caffeic acid phenethyl ester (CAPE) and artepillin C (ARC), the major anti-cancer ingredients in propolis, also causes the exactly same effect on this nematode, suggesting the possibility that the kinase PAK1 might be a new target of Ivermectin. This kinase is required for the growth of more than 70% of human cancers such as pancreatic, colon, breast and prostate cancers and neurofibromatosis (NF) tumors. Here we demonstrate for the first time that Ivermectin blocks the oncogenic kinase PAK1 in human ovarian cancer and NF2-deficient Schwannoma cell lines to suppress their PAK1-dependent growth in cell culture, with the IC_{50} between 5-20 μM depending on cell lines.

Keywords: Ivermectin, PAK1, ovarian cancer, neurofibromatosis type 2 (NF2), *C. elegans*

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1. Introduction

Ivermectin/Avermectin is an antibiotic of macrolide family isolated in 1979 from a filamentous bacterium called *Streptomyces avermitilis* (*1*), and further developed by Merck and Kitasato Institute during mid-1980s as the exceptionally potent and most broad spectrum anti-parasitic drug which kills the intestinal worms (nematodes) in human and several other mammals such as horse and dog at a very low dose (0.2 mg/kg) (*2*). It blocks selectively the worms' GABA (γ-aminobutyric acid) receptor (*3*), but not mammalian counterpart. In 1987 it was found that Ivermectin is a muscle relaxant which blocks the phosphorylation of the regulatory light chain of myosin II (double-headed myosin) in tonic Ascaris suum muscle (*4*), indicating that this drug somehow inhibits a kinase(s) responsible for the phosphorylation of this myosin light chain. The tiny nematode *C. elegans* swallows its food, bacteria, by pharyngeal pumping. In 1990 it was reported that Ivermectin at sublethal doses reduces dramatically the rate of this pumping, leading to a near starvation/fast (*5*), indicating that this drug relaxes the pharyngeal smooth muscle of this nematode. In 2004 a Russian group demonstrated that Ivermectin can suppress the growth of human melanoma and a few other cancer xenografts in mice at the higher doses (3-5 mg/kg) almost completely without any adverse effect on mice (*6*). This prompted us to determine the detailed molecular mechanism underlying its anti-cancer action.

Interestingly, prior to this investigation of ours, an Australian group reported that Ivermectin at sublethal doses reduces the litter size (number of eggs laid) of *C. elegans* by almost 90% (Grant W *et al*., unpublished observation). This phenomenon is almost identical to our own observation with either PAK1-deficiency (in the *C. elegans* strain RB689) or treatment of this nematode with anti-PAK1 drugs such as CAPE (caffeic acid phenethyl ester) and ARC (artepillin C), the major anti-cancer ingredients in NZ (New Zealand) and Brazilian green propolis (*7-9*), suggesting that the major oncogenic kinase PAK1 could be a new target

of Ivermectin. This notion is compatible with the previous observation that the regulatory light chain of myosin II is phosphorylated by PAK1 (*10*). Finally in the study presented here we provide the first direct evidence indicating that Ivermectin indeed inactivates the PAK1 in human ovarian cancer and NF2-deficient Schwannoma cell lines in cell culture, and can suppress the PAK1-dependent growth of these tumor cell lines, with the IC_{50} between 5-20 µM depending on cell lines.

2. Materials and Methods

2.1. *Cell lines and reagents*

Human ovarian cancer cell lines TYK-nu, KOC7c, SKOV3 and RMUG-S as well as human NF2-deficient Schwannoma cell line (HEI-193) were maintained under the standard cell culture conditions as described previously (*8,9*). Human normal embryonic kidney cell line (HEK-293) was obtained from American Type Culture Collection (ATCC). Ivermectin was purchased from Sigma-Aldrich (Cat #18898). Monoclonal antibody against p-Raf1 (Ser 338) was obtained from Cell Signaling (p-c-Raf Ser 338 #9427).

2.2. *Assay for the kinase activity of PAK1 in cell culture*

The phosphorylated level of the kinase Raf1 at Ser 338 (p-Raf1) was monitored by means of the monoclonal antibody against p-Raf1 (×1,000 dilution), as the direct indicator of the kinase activity of PAK1 in ovarian cancer cell lines, TYK-nu and RMUG-S, cultured in the presence or absence of Ivermectin (0-40 µM) for 48 h. Immunoblot analysis was performed as described previously (*9*), and the actin level was used as an internal control for the cellular protein loading at each gel slot.

2.3. *Cell growth inhibition by Ivermectin*

2.0×10^3 cells of ovarian cancer cell lines or 10^3 cells of Schwannoma cell line were seeded per well, and cultured for 3 and 6 days, respectively, in the presence or absence of Ivermectin, at various concentrations and their growth was monitored by MTT method, measuring the optical density at 550 nm as described previously (*8,9*).

3. Results and Discussion

3.1. *Inactivation of PAK1 by Ivermectin*

Since Ser 338 of the kinase RAF1 is the major target site by the phosphorylation of PAK1 and its phosphorylation is essential for the activation of the former (*9,11*), we monitored the kinase activity of PAK1 in each ovarian cancer cell line by measuring the phosphorylation levels of RAF-1 using the antibody specific for the phosphorylated (p)-Raf-1 (Ser 338). As shown in Figure 1, Ivermectin inhibits the phosphorylation of RAF-1 at Ser 338 in cell lines TYK-nu and RMUG-S with IC_{50} around 5 and 20 µM, respectively, clearly indicating that Ivermectin inactivates PAK1 somehow in these cancer cells.

3.2. *Inhibition of growth of ovarian cancer and NF2-deficient Schwannoma cells*

To see whether Ivermectin can suppress the growth of human ovarian cancer cell lines which requires the kinase PAK1 (*12*), we determine the IC_{50} of Ivermectin for their growth. As shown in Figure 2, Ivermectin inhibits the growth of 4 distinct cancer ovarian cell lines including TYK-nu and RMUG-S with IC_{50} around 10 and 20 µM, respectively. Since the IC_{50} of Ivermectin for both PAK1 and growth are roughly the same in each cell line, it is most likely that the growth inhibition of these cell lines is mainly due to the inactivation of PAK1 by Ivermectin.

To further confirm this notion, we have tested the effect of Ivermectin on the growth of NF2-deficient Schwannoma cell line (HEI-193) which has been established to require the kinase PAK1 and inhibited by a variety of anti-PAK1 drugs such as CEP-1347,
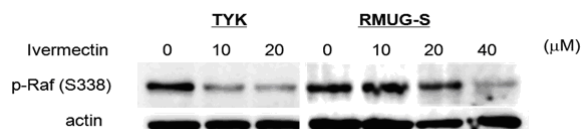

Figure 1. Ivermectin down-regulates the phosphorylation of Raf1 at Ser 338 (p-Raf1) by inactivating the kinase PAK1 in two different ovarian cancer cell lines, TYK-nu and RMUG-S. The p-Raf1 levels were estimated by the monoclonal antibody against p-Raf1 as described under "Materials and Methods".
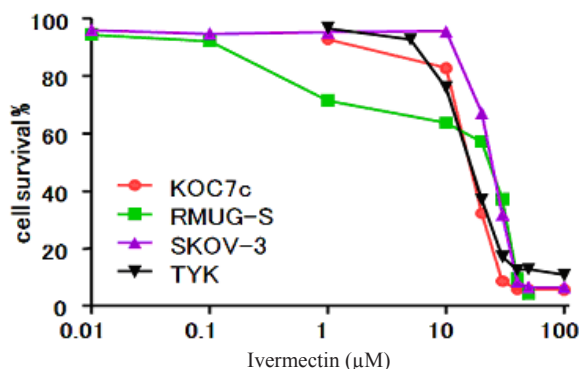

Figure 2. Ivermectin inhibits the growth of four different human ovarian cancer cell lines at the indicated concentrations. For detail, see "Materials and Methods".
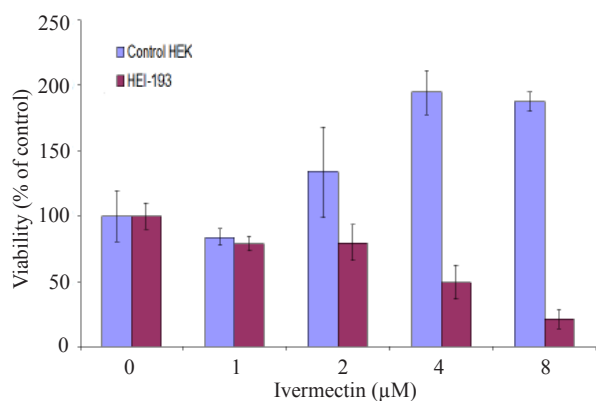
Figure 3. Ivermectin inhibits the growth of human Schwannoma cell line HEI-193, but not the normal cell line HEK-293. Statistical analysis indicates a significant difference in the viability between the control HEK-293 and HEI-193 at 4-8 μM of Ivermectin ($p < 0.001$).

FK228, CAPE, and ARC (*8,9,13,14*). The NF2 gene product called Merlin is a direct inhibitor of PAK1, and therefore in the NF2-deficient cell lines, PAK1 is abnormally activated (*13,14*). As shown in Figure 3, the PAK1-dependent growth of this Schwannoma cell line was selectively inhibited by Ivermectin with the IC_{50} around 5 μM, whereas Ivermectin at these tested concentrations showed no toxicity on the control normal human embryonic kidney cell line (HEK-293) at all, confirming again that PAK1 is not essential for the normal cell growth. These data altogether strongly suggest that Ivermectin would be potentially useful for the treatment of both ovarian cancers and NF2 tumors as well as many other PAK1-dependent cancers such as pancreatic, colon, gastric, breast, prostate, cervical, lung, and thyroid cancers, melanoma, MM (multiple-myeloma), glioma, hepatoma, and NF1-deficient cancer such as MPNST (*12-19*).

As the effective therapeutics for these PAK1-dependent cancers or tumors, only the bee products "propolis" such as CAPE-based Bio 30 and ARC-based Brazillian green propolis extract (GPE) have been available on the market. Furthermore, Ivermectin turned out to be the sole chemically defined anti-PAK1 drug which is available on the market inexpensively so far. Although Bio 30 is the most effective and inexpensive, it causes an allergic reaction to 1-2% of population, whereas GPE causes no allergic reaction, but is far more expensive and less effective than Bio 30 (*8,9*). Thus, Ivermectin could be an inexpensive alternative of Bio 30 for those who are allergic to CAPE in Bio 30. The reasonably low price (around a dollar or so for daily treatment) is in particular important for NF and TSC (Tuberous Sclerosis) tumor patients, because these rare disorders are genetic diseases and their treatment which often starts in early childhood would be life-long, unlike cancer treatment which usually starts at much older ages and lasts for only a short time period.

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