Characterization of Rac inhibitors HV-107 and HV-118 as a treatment for triple negative metastatic breast cancer

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Abstract

Among all cancers worldwide, breast cancer is the first cause of death in women. One of the most aggressive subtypes of breast cancer is the triple negative form, which account for the 10-15% of breast cancer cases. Due to its complex heterogeneity triple negative breast cancer available treatments are limited, being chemotherapy in the first line of use. Despite advances in the search for new treatments, there is still a lack of effective therapies, being the metastatic disease the principal cause of breast cancer mortality. Therefore, it is critical to develop a new and effective strategies to inhibit metastasis. The Rho GTPase Rac has been identified as a promising target for anti-metastatic cancer therapy as it has been shown to play key roles in metastatic cancer cells dynamics as: cellular adhesion, migration, proliferation, survival and invasion. To this extent, in an effort to find a compound with increased Rac inhibitory capacity our group developed Ehop-016 derivatives HV-107 and HV-118. Previously we have reported HV-107 and HV-118 to affect cell viability promoting a G2-M cell cycle arrest with a sub-G1 population indicative of cell death in MDA-MB-231. Also, an increase of caspases activity validating apoptosis as a mechanism of cell death was shown. In addition, a decrease in tumor growth and metastasis by ~35 to 40% in an in vivo study using HV-118 at 1 mg/kg body weight was also reported. Currently, through pulldown assays we have showed HV-107 and HV-118 to inhibit Rac activity at~500-2000 nM and 10 nM respectively in MDA-MB-231 and MDA-MB-468 breast cancer cells. Using trypan blue excision assay HV-107(>500 nM) and HV-118(>50 nM) were shown to affect cell viability promoting a G2-M cell cycle arrest in MDA-MB-468. We also demonstrated HV-107 and HV-118 to inhibit the direct downstream effector of Rac, PAK at >500 nM(HV-107) and >50 nM(HV-118) in MDA-MB-231 cells. In addition, HV-107 was also shown to increase Rho activity at concentrations significantly higher than the effective for Rac inhibition. Rho activity up-regulation has been reported to negatively affect migration of cancerous cells. Finally, we tested HV-107 in a mouse model of metastatic breast cancer. A decrease of ~ 40% in liver metastasis was shown for mice treated with 5 mg/kg BW HV-107. Taken together, our results indicate HV-107 and HV-118 are approximately 4-100 times more efficient than the parent compound Ehop-016 and have potential as anti-breast cancer metastasis therapeutics. This study is supported by NIH Grant 1SC1GM122691, and PRINBRE P20GM103475 from NIGMS of the NIH to GVC.

Rac inhibitors HV-107 and HV-118

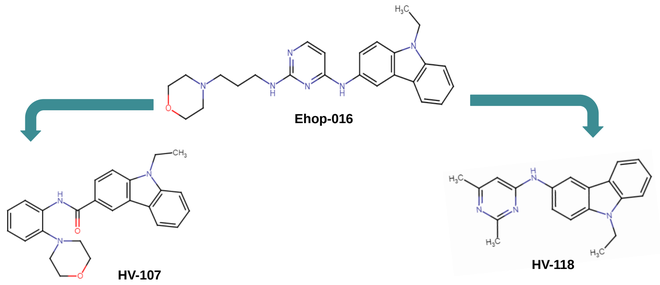


Figure 1. Ehop-016 derivatives HV-107 and HV-118. HV-107 and HV-118 were designed using as a base compound Ehop-016. Pyrimidine core and carbazole group were shown to be important to preserve the affinity of the inhibitors with Rac-guanine nucleotide exchange factors binding pocket.

Rac inhibitors mechanism of action

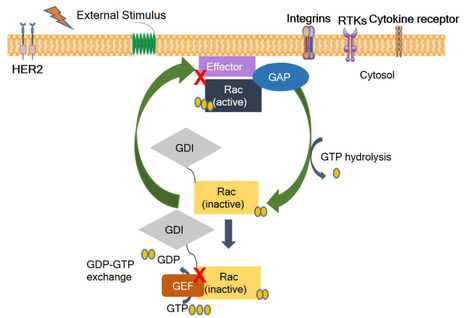
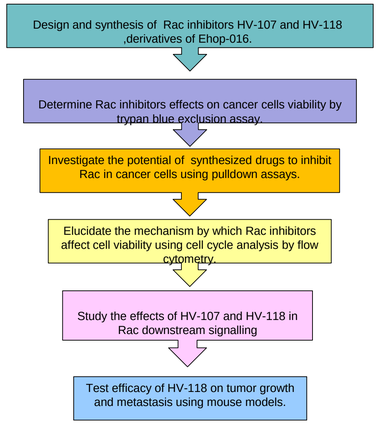


Figure 2. HV-107 and HV-118 mechanism of inhibition. HV-107 and HV-118 interact with the Rac guanine nucleotide exchange factors (GEFs) binding pocket, thus inhibiting the interaction of Rac with its upstream activators. This in turn also affect the activity of Rac downstream effectors involved in important cell dynamics for cancer progression and metastasis as cell proliferation, migration and invasion.

Approach



Inhibition of Cell Viability

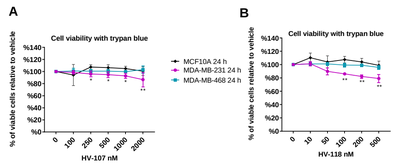


Fig.3 Effects of HV-107 and HV-118 on cell viability. Cells were treated with 0-2000 nM HV-107 or 0-500nM HV-118 for 24h. Trypan blue excision assay was performed for the analysis of cell viability. Relative cell viability is presented for MCF10A, MDA-MB-231 and MDA-MB-468 cells treated with **A.** HV-107 and **B.** HV-118. N=3-4. Error bars = ± SEM; * p≤0.05.

Inhibition of Rac Activation

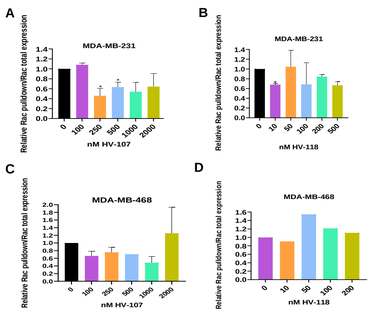


Figure 4. Effect of HV-107 and HV-118 on Rac activation. MDA-MB-231 and MDA-MB-468 triple negative breast cancer cells were treated with **A,C** HV-107(0-2000 nM) and **B,D** HV-118 (0-500 nM) for 24 h. After treatment, total protein was extracted and equal amounts of proteins were subjected to pulldown assays using the p21-binding domain of PAK to isolate the active Rac (GTP bound). Samples were then western blotted for total and active Rac and positive bands quantified using image studio software. N=1-3; error bars represent ±. SEM; * p≤0.05

Increase of Rho Activity

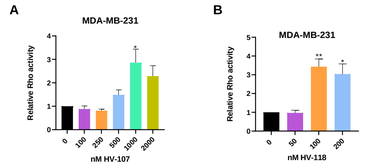


Figure 5. Effect of HV-107 and HV-118 on Rho activation. . MDA-MB-231 cells were treated with **A**,HV-107(0-2000 nM) and **B**, HV-118(0-200 nM) for 24h. After treatment, total protein was extracted and equal amounts of proteins were subjected to pulldown assays using the RBD domain of Rock to isolate the active Rho (GTP bound). Samples were then western blotted for total and active Rho and positive bands quantified using image studio software. N=2-5; error bars represent ± SEM; * p≤0.05.

Effects on Rac downstream signaling

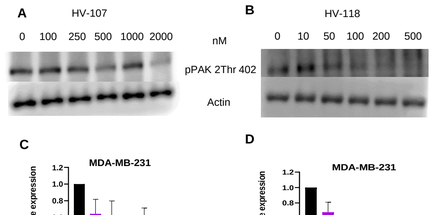


Figure 6. Inhibition of PAK2 phosphorylation by HV-107 and HV-118. MDA-MB-231 cells were treated with **A**,HV-107(0-2000 nM) and **B**, HV-118(0-500 nM) for 24h. After treatment, total protein was extracted and equal amounts of proteins were western blotted for phosphorylated PAK 2; positive bands (**A,B)** were quantified (**C,D**) using image studio software. N=2-3; error bars represent ± SEM; * p≤0.05.

Effects on cell cycle

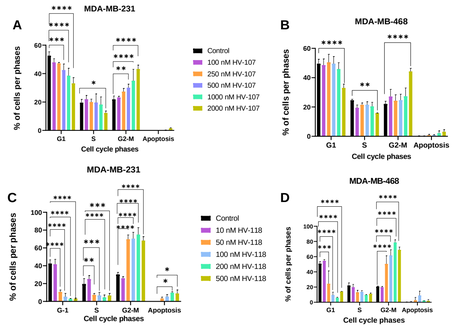


Figure 7. Effects of HV-107 and HV-118 on cell cycle. A,C,MDA-MB-231 and **B,C**,MDA-MB-468 were treated with 0-2000 nM HV-107 or 0-500 HV-118 for 24 hr. After treatment cells were collected, washed and resuspended. Equal amounts of cells were fixed with 70% ethanol, and stained with propidium iodine. Samples and results were analysed using Guava easy Cyte flow cytometer (Luminex) and InCyte software. N=3-4; graphs were generated using means and error bars represent ± SEM; * p≤0.05.

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In vivo mice study

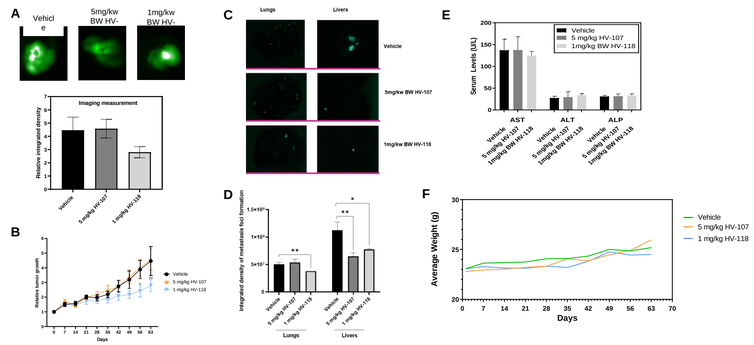

Figure 7. Effect of HV-107 and HV-118 on metastasis formation. Severe combined immune deficiency (SCID) mice were inoculated at the mammary fat pad with GFP-MDA-MB-231 cells and treated with 0,5 mg/kg body weight (BW) HV-107 or 1 mg/kg BW HV-118 (5x a wk). **A, *upper panel*** representative tumors under fluorescent microscopy for 0, 5mg/kg body weight (BW) HV-107 or 1mg/kg BW HV-118.**A, *lower panel*** relative tumor growth quantitation, N=13-15 mice/group, error bars represent ± SEM; * p≤0.05. **B**, relative mouse tumors growth were measured as a function of days. **C**, mice were treated with 0, 5 mg/kg BW HV-107 or 1mg/kg HV-118 BW and at necropsy lungs and livers were removed and imaged for metastatic foci. **D,** Relative integrated density fluorescent of metastatic foci/organ. **E,** Serum levels of hepatic enzymes as measure of toxicity. **F,** Mouse weights were measured as a function of days and used as an indirect evaluation of toxicity.

HV-107 and HV-118 disruption of Rac signaling

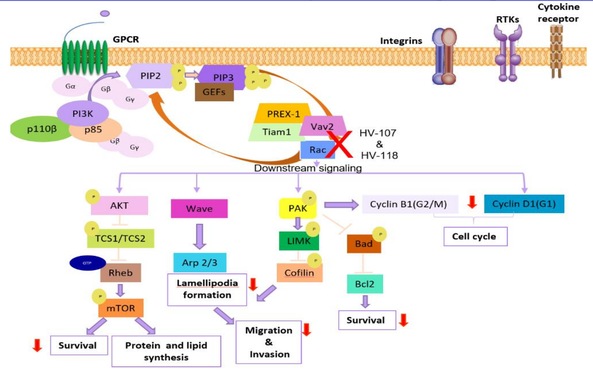

Figure 8. Effects of HV-107 and HV-118 on Rac signaling. Rac inhibitors HV-107 and HV-118 inhibit the interaction of Rac with its upstream activators (GEFs) thus disrupting the Rac downstream effectors activation. The downstream signaling of Rac include cyclins for cell cycle progression (proliferation), pro-apoptotic signalling inhibition(survival) and signalling for cytoskeleton rearrangement related to cell migration and invasion.

Summary and Conclusions

- HV-107 and HV-118 significantly inhibits the viability of MDA-MB-231 and MDA-MB-468 triple negative metastatic cell lines while showing low toxicity towards non-cancerous mammary epithelial cells.
- Rac activity is significantly inhibited (~30-40%) by HV-107 and HV-118 in both MDA-MB-231 and MDA-MB-468 triple negative metastatic breast cancer cell lines.
- HV-107 and HV-118 decreases PAK2 phosphorylation by 50 to 80%, suggesting that both HV-107 and HV-118 affect the downstream signalling of Rac.
- HV-107 and HV-118 affect cell viability promoting a G_2-M cell cycle arrest along with a prominent sub G_1 cell population, indicative of cell death.
- HV-107 at 5 mg/kg BW inhibit liver metastasis by~ 50% whereas HV-118 at 1 mg/kg BW decrease tumor growth and inhibit lung and livers metastasis by 30% and 45% respectively.
- Both HV-107 and HV-118 showed no toxicity on in vivo mice study.

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