

## Different responses of HepG2 subclones to low dose ethaselen

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**Abstract:** As a synthesized antineoplastic organoselenium compound, ethaselen is known to induce apoptosis in tumor cells via dose-dependent thioredoxin reductase (TrxR) inhibition. Thioredoxin, the multifunctional biological substrate of TrxR, is then left in the oxidized state, which subsequently leads to intracellular accumulation of reactive oxygen species (ROS), cell cycle arrest and/or apoptosis. However, the low dose effect of ethaselen remains largely unknown. Several subclones have been derived from HepG2 cells by using single cell or colony isolation. The low dose of ethaselen was defined as the drug concentration of retaining >90% HepG2 cells alive. The HepG2 cells were used as reference of its subclones (SM01, SM02 and SM03), and the cell cycle transition, intracellular proteins change, colony formation and sphere growth were assayed in treatment of low dose ethaselen. HepG2 and its subclones differently responded to lethal dose of cisplatin or 5-fluorouracil. Low dose of ethaselen (1  $\mu$ M) modulated the cell cycle transition at 12 h of treatment, but cells were partially recovered at 24 h of treatment though some proteins were still affected. Low dose of ethaselen did not inhibit the small colony (diameter >100  $\mu$ m) formation and sphere growth of HepG2 and SM01. However, low dose of ethaselen could specifically inhibit the survival, large colony (diameter >500  $\mu$ m) formation and sphere growth of SM03, although SM03 could be rapidly recovered from ethaselen-induced cell cycle check. HepG2 and its subclone cells could survive but respond differently to treatment of low dose ethaselen (1  $\mu$ M). Low dose of ethaselen could significantly inhibit a HepG2 subclone (SM03) in cell survival and colony growth.

**Keywords:** Low dose, Organoselenium, Cell subcloning, Heterogeneity, Tumor cell

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### 1. Introduction

As a synthesized antineoplastic organoselenium compound, ethaselen can induce apoptosis in approximate a dozen of cancer cell lines in vitro and in vivo<sup>[1-7]</sup>. Based on its specific inhibition of thioredoxin reductase (TrxR) in isolated redox system of TrxR and its biological substrate thioredoxin (Trx), it has been further revealed that ethaselen can specifically inhibit cytoplasmic TrxR1 (maybe also TrxR2), which subsequently leads to the redox balance disturbance, reactive oxygen species (ROS) accumulation and eventually mitochondria-dependent apoptosis<sup>[4,8,9]</sup>. In addition, it has been reported that ethaselen can cause cell cycle arrest<sup>[1,7,10]</sup>, participate in immune response modulation<sup>[11]</sup> and enhance the

cytotoxicity of cisplatin and selenite<sup>[6,12]</sup> and effect of radiation<sup>[13]</sup>. All these evidence of ethaselen implied that this chemical acts via multiple intracellular mechanisms although predominantly via targeting multifunctional TrxR-Trx system<sup>[7]</sup>. By inhibiting TrxR, ethaselen displays a time- and dose-dependent effect of cell growth inhibition, cell cycle arrest and apoptosis induction. To investigate an independent biological effect and mechanism of ethaselen treatment other than TrxR inhibition, we performed a series of cell-based studies under the concentration that was not favorable in the range of TrxR inhibition. Moreover, the low dose effect of ethaselen has not been explored.

An obvious obstacle in studying the low dose effects of drugs on tumor cells is its heterogeneous cell components even with isolated cell lines. It is now accepted that the stem cells endow the cell population with limitless capacity of proliferation. These stem cells are also able to differentiate into different functional and morphological daughter cells with or without induction<sup>[14]</sup>. Hepatocellular

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carcinoma (HCC) represents the tumor of complicated heterogeneity, and existing chemotherapeutic drugs are ineffective in clinical treatment. HCC patients are usually subjected to surgical resection or liver transplantation, but most of them are not suitable any longer when diagnosed<sup>[15,16]</sup>. By isolating the colony derived from a single cell, several HepG2 subclones have been demonstrated to contain different characteristics from their parental cell<sup>[17]</sup>. We hypothesized that if these subclones would represent various cell components of HepG2 cell line, they were able to display different responses to treatment of low dose ethaselen. In this study, we defined the low dose concentration of ethaselen based upon IC<sub>50</sub> value, which was further validated by cell cycle transition, intracellular protein change, colony formation and sphere growth of multiple HepG2 subclones.

## 2. Materials and methods

### 2.1. Materials

Ethaselen (1,2-[bis(1,2-benzisoselenazolone-3(2*H*)-ketone)]-ethane) in DMSO (20 mM stock solution) (formerly also abbreviated as BBSKE, PCT: CN02/00412), butaselen (1,2-[bis(1,2-benzisoselenazolone-3(2*H*)-ketone)]-butane) in DMSO (20 mM stock solution) and pentaselen (1,2-[bis(1,2-benzisoselenazolone-3(2*H*)-ketone)]-pentane) in DMSO (20 mM stock solution) were synthesized and prepared in our laboratory. Cisplatin (USP32 99%, Italo Biological Technology, China), 5-fluorouracil (Sigma, Cat. # F6627), MTT (Sigma), agar (Sigma, Cat. #A1296), propidium iodide (Sigma), RNase A (Amersco), mouse anti-p53 (Santa Cruz, Cat. #sc-126), rabbit anti-NK-κB p50 (sc-114), rabbit anti-IκB-α (sc-371), mouse anti-p-JNK (sc-6354), mouse anti-β-catenin (sc-7963); mouse mAb TrxR2 IgG1 (sc-365714), mouse anti-actin mAb (ZSGB, Cat. #TA-09), BCA reagent (Applygen, China), and Amersham ECL prime Western Blotting detection reagent (GE Healthcare, Cat. # RPN2232); and equipments and reagents for conventional cell culture.

### 2.2. HepG2 cell cloning

HepG2 cell line was introduced from Chinese Academy of Medical Science and maintained in RMPI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Excell, China), 100 U/mL penicillin and 100 μg/mL streptomycin. The cell cloning was performed as previously described<sup>[18]</sup>. Briefly, monolayer HepG2 cells were trypsinized and counted with hemacytometer, cell suspension was serially diluted to 10 cells/mL, and 100 μL cell suspension was added to 96-well plate and marked the wells with single cell. The plate was maintained in a humidity-controlled incubator with supply of 5% CO<sub>2</sub> for one week. The colony containing more than 100 cells was isolated and sequentially proliferated until more than 10<sup>5</sup> cells were obtained. Using this method, 13 clones were amplified, named as M01–M13. In soft-agar clone isolation, 100-fold concentrated cell suspension was prepared, mixed with pre-warmed 0.3% agar (in complete medium) and seeded as upper layer of soft-agar colony formation assay. After culture for 2–3 weeks, the colonies of diameter >100 μm were transferred into 24-well plate for further proliferation until cell number was greater than 10<sup>5</sup> cells. By using soft-agar colony formation assay, three HepG2 sub-clones were isolated, named SM01, SM02 and SM03.

### 2.3. Cytotoxic test

HepG2, its monolayer subclones (M01–M11, M13) and its soft-agar subclones (SM01, SM02 and SM03) were subjected to lethal dose of chemicals. Cell suspension (2×10<sup>3</sup> cells/100 μL) was seeded in 96-well plate for 48 h. The medium was refreshed with 180 μL complete medium plus 20 μL chemicals (final concentration of 20 μg/mL cisplatin or 5-fluorouracil). After 24-h exposure, the chemical was removed, and cells were further cultured for 48 h prior to MTT staining. In order to estimate the half cell population inhibitory concentration (IC<sub>50</sub>), two thousand cells were seeded for 48 h before 72-h ethaselen treatment. After removal of chemicals, the cells were recovered for 24 h or 48 h prior to MTT

staining. Four-parameter logistic model was used to estimate the IC<sub>50</sub> of ethaselen.

#### 2.4. DNA content analysis

DNA content was quantified as previously described<sup>[17]</sup>. Briefly, after the trypsinized cells were pelleted and washed twice with D-PBSA, the cell suspension was adjusted to  $<10^6$  cells/500  $\mu$ L and fixed with cold ethanol overnight. The fixed cells were then washed twice with D-PBSA + 1% BSA and treated with PI and RNase A at 37 °C for 10 min, followed by filtration of 40- $\mu$ m mesh cell strainer. A total of 5000 cells per sample were analyzed by using Guava Easycyte Plus system (Millipore, Billerica, MA), and the cell cycle distribution was calculated with ModFit LT 3.2 software.

#### 2.5. Western blotting

The protein immunoblotting was performed as previously described<sup>[17]</sup>. After cells were lysed with RIPA + 1 mM PMSF (just before use) solution for 30 min, the supernatant was collected, followed by a 30-min centrifugation at 12 000  $\times$ g, 4 °C. The proteins in supernatant were quantified with BCA reagent, diluted with 5 $\times$  Laemmli buffer and denatured. A total of 20  $\mu$ g proteins were loaded in each lane of PAGE gel. After the proteins were separated, they were then trans-blotted onto PVDF membrane. The blots were blocked with 5% skimmed milk and then incubated with antibodies against p53 (1:2000), NK- $\kappa$ B (1:5000), p-JNK (1:2000), TrxR2 (1:5000), I $\kappa$ B (1:5000),  $\beta$ -catenin (1:1000) and  $\beta$ -actin (1: 5000) in TBST +1% BSA at 4 °C overnight, followed by incubation with secondary antibodies at room temperature for 1 h. Finally, the bands were developed with ECL reagent and recorded with Kodak X-ray film.

#### 2.6. Sphere culture

The HepG2 cell spheres that were initiated and maintained in continuous, static suspension culture were performed as previously described<sup>[17]</sup>. The suspended HepG2 cells formed spheres in 4–6 d, and the suspension culture of spheres was passaged every 5–7 d according

to the density and growth velocity. These spheres were picked out and individually seeded onto 1% agar-coated 96-well plates. As the spheres did not dissociate with enzyme or mechanical force, the continuity of its extracellular matrix could be largely retained.

#### 2.7. Statistics

The means were compared with student's *t* test, and proportional data were compared with *chi*-square test or Fisher exact test. R software (Version 2.15.2) was used in statistical analysis and cytotoxic assay curve fitting. The two-tailed *P* value  $<0.05$  was considered statistically significant.

### 3. Results

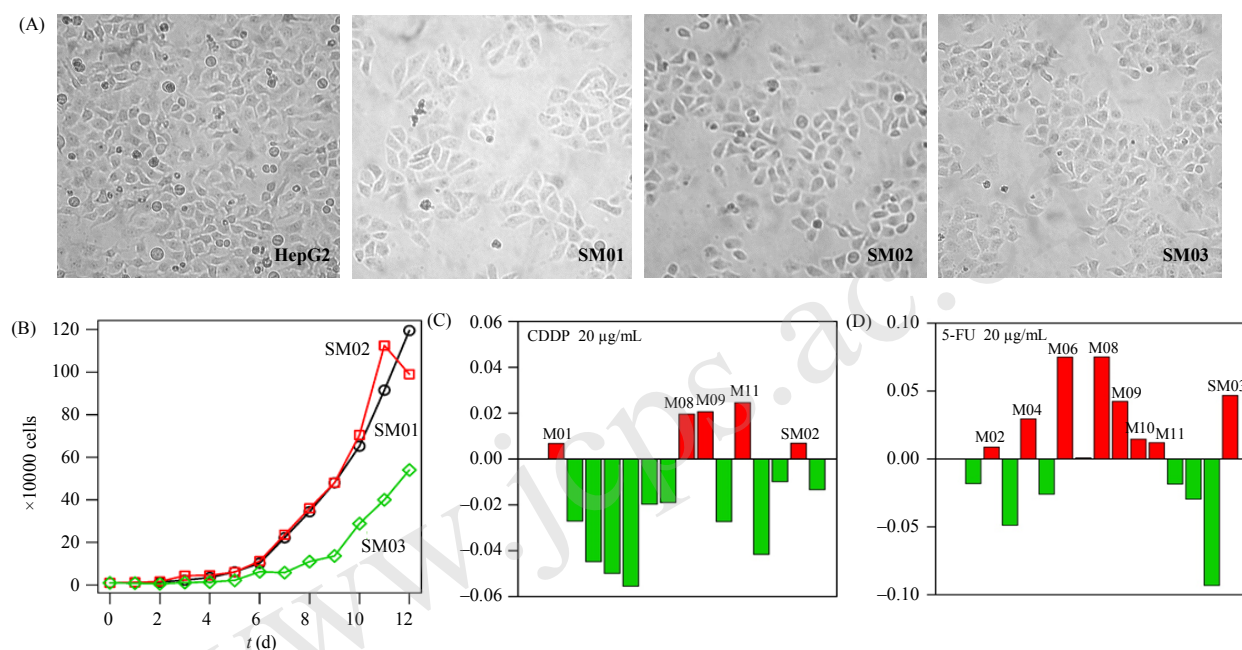
#### 3.1. HepG2 subclones heterogeneously respond to ethaselen

It has been previously indicated that the soft-agar subclones of HepG2 (SM01, SM02 and SM03) contain different characteristics from their parental cells in terms of secondary colony formation capability, cell cycle distribution and sensitivity to chemicals (Fig. 1A). The growth rate of SM03 was slower than that of SM01 and SM02 (Fig. 1B). In addition, SM01, SM02, SM03 and subclones from single monolayer cell (cell images not present) displayed heterogeneous response to the lethal dose of cisplatin or 5-fluorouracil. By referring to HepG2, subclones of M03, M04, M05 and M13 were more sensitive to lethal dose of cisplatin, displaying  $>4\%$  inhibition, while M01, M08, M09, M11 and SM02 indicated a marginal resistance (Fig. 1C). M06, M08, M09 and SM03 were more resistant to lethal dose of 5-fluorouracil, while M03 and SM02 were more sensitive (Fig. 1D). The heterogeneous response of HepG2 subclones was also detected in ethaselen cytotoxic assay, especially in low dose range (Fig. 2A). These subclones heterogeneously responded to relative low concentration of ethaselen, suggesting a unique mechanism of ethaselen.

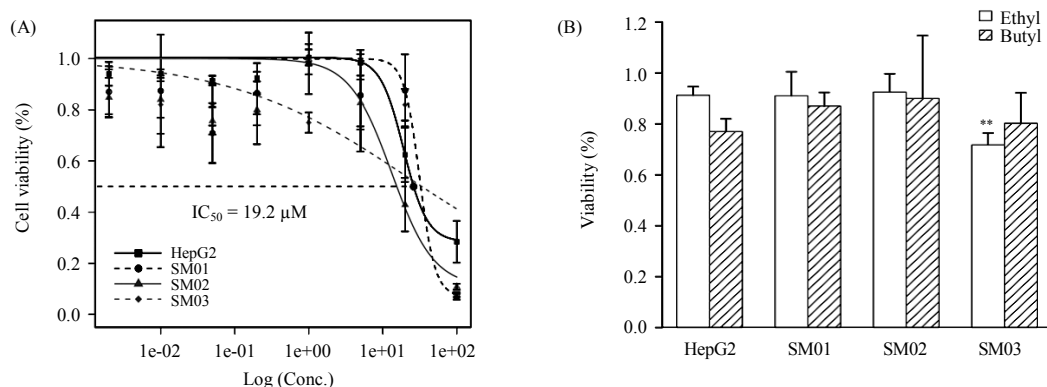
### 3.2. Definition of low dose of ethaselen

The low dose of ethaselen was defined using their parental, HepG2 cell. Based upon the  $IC_{50}$  value for HepG2, 1  $\mu$ M ethaselen was estimated to kill around 5%–10% cells in 72-h treatment, while the majority of cells could survive (Fig. 2A). Therefore, 1  $\mu$ M ethaselen was defined as low dose in this study, and HepG2 was

used as reference for its subclones. This effect of 1  $\mu$ M ethaselen was further confirmed with its analogue, butaselen, except that the SM03 was significantly sensitive to ethaselen compared with SM01, SM02 and HepG2 (Fig. 2B). The functional groups of butaselen (and pentaselen) were the same as ethaselen, where the linking arms of ethane were replaced by butane (and pentane).



**Figure 1.** Subclones of HepG2 were heterogeneous. (A) HepG2 and its three soft-agar derived subclones (SM01, SM02 and SM03) as monolayer, 100 $\times$ ; (B) three soft-agar derived subclones indicated different growth velocity as monolayer; (C) HepG2 subclones responded to lethal dose of cisplatin (20  $\mu$ g/mL CDDP) for 24-h treatment; the first bar is HepG2 (reference), subsequently followed M01, M02, M03, M04, M05, M06, M07, M08, M09, M10, M11, M13 (derived from monolayer single cell) and SM01, SM02, SM03 (derived from soft-agar single colony); (D) HepG2 subclones responded to lethal dose of 5-fluorouracil (20  $\mu$ g/mL 5-FU) for 24-h treatment, the sequence of HepG2 and its subclones is the same as above.



**Figure 2.** The definition of low dose ethaselen. (A) The estimated  $IC_{50}$  of ethaselen was 19.2  $\mu$ M for HepG2 for 72-h treatment and 48-h recovery, 1  $\mu$ M was estimated to kill about 5%–10% of HepG2 cells; after 72-h treatment, SM01, SM02 and SM03 were recovered for 24 h before staining; (B) The percentage of viability after ethaselen (1  $\mu$ M) and its analogue butaselen (1  $\mu$ M) treatment; \*\* $P$ <0.01 compared with HepG2, SM01, SM02.

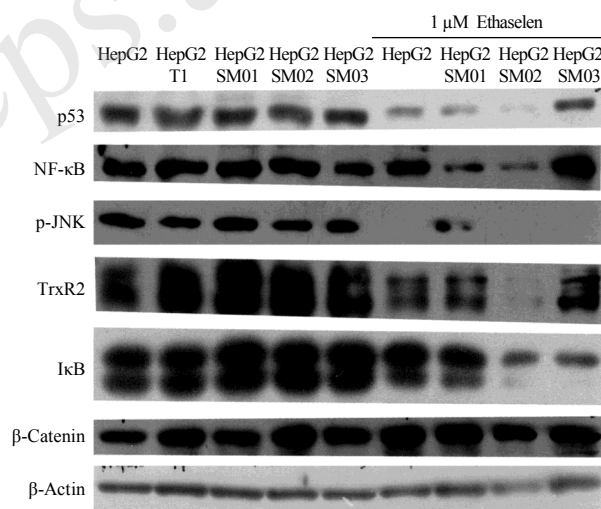
### 3.3. Low dose of ethaselen affects cell cycle transition

Since tumor cells have a doubling time of around 24 h, the cell cycle transition could be rapid. Therefore, the cell cycle transition might be affected by the chemicals with instant cell growth inhibition. After seeding for 12 h, SM01, SM02 and SM03 showed a higher cell population proportion in S phase compared with HepG2. However, this trend was attenuated at 24 h after seeding. When treated with 1  $\mu$ M ethaselen for 12 h, more HepG2 cells were arrested in the S phase. However, under this condition, SM01, SM02 and SM03 displayed a different cell cycle distribution profile compared with HepG2 (Table 1). A transient cell cycle arrest of HepG2 and its subclones was observed by the treatment with low dose of ethaselen. Moreover, the underlying protein levels of HepG2 and its subclones were also affected by the treatment with low dose of ethaselen, especially in SM01 and SM02 subclones (Fig. 3). However, the protein levels of HepG2 were not significantly affected by the treatment with low dose of ethaselen, suggesting that the HepG2 cell line could manage to overcome the drug effect. Further studies are required to focus on the inhibitory effect of long-period treatment of low dose ethaselen in tumor growth and cell cycle progression.

### 3.4. Low dose of ethaselen affects sphere growth

SM01, SM02 and SM03 were known to have elevated secondary colony forming capacity compared with HepG2 cells; while this capability would be gradually lost when proliferating as monolayer. To study the effect of low dose ethaselen in colony formation, ethaselen (1  $\mu$ M) was co-administered with cell seeding

in soft-agar assay. After two weeks, the colony numbers of diameter >100  $\mu$ m were comparable between untreated and treated groups. Pentaselen, an analogue of ethaselen, was also applied in this study, and a similar response pattern as ethaselen was observed (Fig. 4A). When comparing the colony formation response of suspension culture from monolayer, the monolayer SM03 cells were sensitive to treatment of low dose ethaselen (Fig. 4B). Overall, the colony formed from single cell proliferation was not significantly affected by the treatment with low dose of ethaselen. However, the results from subsequent study suggested that the sphere growth might be modulated (Fig. 4C, Table 2). Low dose of ethaselen was able to impede the SM03 sphere growth during a 12-day exposure, but such finding was not detected in SM01 or SM02.

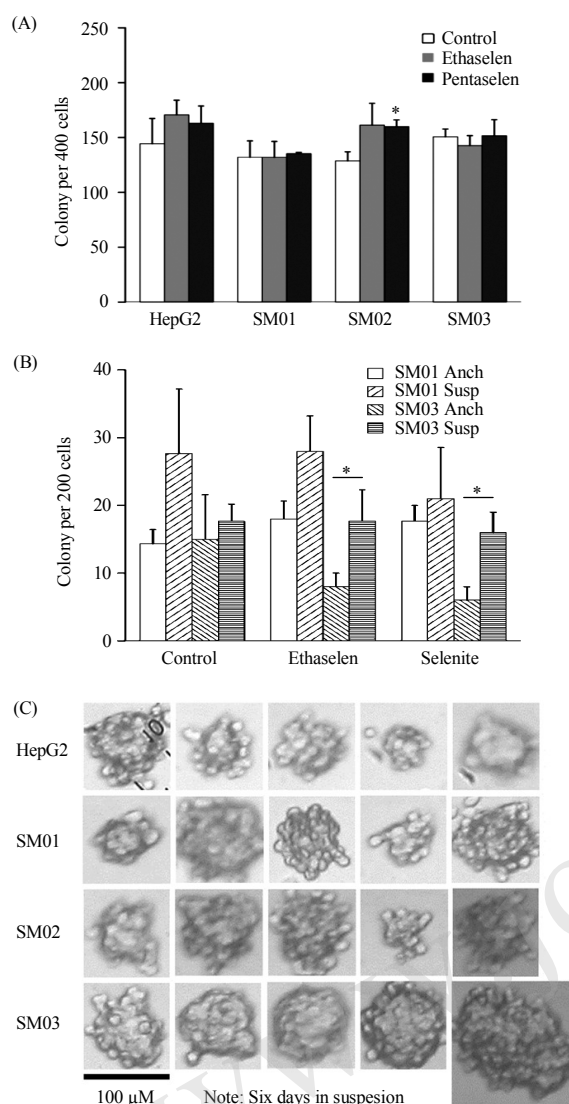


**Figure 3.** Proteins recovered from 24-h ethaselen (1  $\mu$ M) treatment; HepG2 T1 cells were derived from HepG2 tumor in nude mice, here used as a reference of HepG2.

**Table 1.** Cell cycle analysis

	HepG2				SM01					SM02					SM03				
	12 h	24 h	E12 h	E24 h	0 h	12 h	24 h	E12 h	E24 h	0 h	12 h	24 h	E12 h	E24 h	0 h	12 h	24 h	E12 h	E24 h
Dip G1, %	58.9	56.8	38.4	55.3	44.1	55.0	58.3	58.0	56.8	39.2	49.8	61.1	57.6	64.1	49.3	51.7	53.5	55.1	51.0
Dip S, %	28.6	31.2	59.0	32.6	48.6	42.9	34.6	41.9	38.3	46.7	49.5	23.6	42.4	35.9	47.7	45.1	40.7	44.7	36.6
Dip G2, %	12.4	12.0	2.6	12.1	7.3	2.1	7.2	0.0	4.8	14.1	0.7	15.3	0.0	0.0	3.0	3.2	5.7	0.2	12.3
CV, %	28.6	18.9	18.4	20.0	8.2	7.8	14.8	12.6	19.7	8.9	8.3	20.4	14.9	14.1	9.0	11.6	21.1	15.0	18.2
G2/G1	NA	NA	NA	NA	2.0	NA	NA	NA	NA	2.0	2.0	NA	NA	NA	2.0	NA	NA	NA	NA
$\chi^2$ test			##			**		**			**		**			**		*	

E12 h, ethaselen 1  $\mu$ M 12 h treatment; E24 h, ethaselen 1  $\mu$ M 24 h treatment; \* $P$ <0.05; \*\* $P$ <0.01 compared with those of HepG2; ## $P$ <0.01 compared with HepG2 12 h.



**Figure 4.** Ethaselen of low dose affects large colony formation and sphere growth of HepG2 cells and its subclones. (A) After passaging in vitro, SM01, SM02 and SM03 did not indicate higher colony-forming capacity compared with HepG2 with respect to colonies of diameter  $>100 \mu\text{M}$ ; ethaselen ( $1 \mu\text{M}$ ) did not inhibit the small colony formation; (B) Both ethaselen ( $1 \mu\text{M}$ ) and selenite ( $1 \mu\text{M}$ , as reference) treatment for three weeks inhibited monolayer SM03 colony formation (with diameter  $>500 \mu\text{M}$ ); (C) The spheres formed in static, continuous suspension culture and used in single sphere tracing.

**Table 2.** Sphere inhibition rate of  $1 \mu\text{M}$  ethaselen

Cell populations	Sphere number of inhibition (%)	Sphere number of seeding	P value
HepG2	28 (5.7)	480	Reference
HepG2 + Ethaselen	13 (6.8)	192	0.779*
SM01	25 (26.0)	96	$<0.0001$
SM01 + Ethaselen	24 (25.0)	96	$1.0^*$
SM02	71 (37.0)	192	$<0.0001$
SM02 + Ethaselen	103 (53.6)	192	0.0015*
SM03	76 (39.6)	192	$<0.0001$
SM03 + Ethaselen	59 (30.7)	192	0.0115*

Single spheres were traced for 12 d; SM01, SM02 and SM03 indicated elevated sphere response rates compared with their parental cell; \*comparison between with and without  $1 \mu\text{M}$  ethaselen treatment.

#### 4. Discussion

Overall, these subclones demonstrated different responses to lethal dose of cisplatin and 5-fluorouracil, and low dose of ethaselen in cell cycle transition, colony formation and sphere growth. Specifically, subclones (SM01 and SM02) shared a similar cellular morphology and response to 5-fluorouracil. Moreover, the cell cycle transition profile and the underlying change of p53 and NF- $\kappa\text{B}$  levels were also consistent between SM01 and SM02, suggesting that these two subclones differed from SM03. Therefore, we used only SM01 and SM03 to compare the different effects of suspension culture with monolayer. We also noticed the varied responses of different subclones in cell survival, colony formation and sphere growth to treatment of low dose ethaselen. However, further study should clarify to what extent these subclones share characteristics with their parental cell. These data demonstrated the different responses of these HepG2 subclones to multiple stimuli, however, its underlying molecular mechanism remains unclear.

Low dose effect of chemicals is of great importance as it is generally considered to imitate the tumor cell response to circulating drugs in clinical practice. The low dose of ethaselen defined in this study was not only indicated as its  $\text{IC}_{50}$  of HepG2 for 72-h exposure, but also validated as the 1/5–1/10 of previously reported effective concentrations<sup>[1,3,8]</sup>. In a previous study, the estimated  $C_{\text{max}}$  following oral administration of ethaselen at a dose of 30 mg/kg body weight in beagle dogs is  $(0.51 \pm 0.28) \mu\text{g/mL}$  in plasma<sup>[19]</sup>. The molecular weight of ethaselen and its analogues were all less than 500 Da. The  $C_{\text{max}}$  level in beagle dog was higher than  $1 \text{ nmol/mL}$  (i.e.,  $1 \mu\text{M}$ ). If we speculated that this animal study could provide a comparable drug kinetic model for humans, the concentration of ethaselen in human plasma could reach  $1 \mu\text{M}$ . The liver and spleen have been also reported to retain a portion of this drug in 7 d<sup>[2]</sup>. Due to the low toxicity of this compound, the doses of ethaselen can be further escalated in clinical practice. In addition, structural modification based upon ethaselen backbone has obtained more potential candidates<sup>[20]</sup>. This low dose definition was not unique among different

cell lines, and the estimated IC<sub>50</sub>s are in the range of 5–40  $\mu\text{M}$ <sup>[1–3,5]</sup>. Therefore, a minor adjustment of concentration is required to study the low dose effect of ethaselen for other cancer cell lines. This study provided a feasible model in definition and analysis of low dose effects of ethaselen.

Based on our data, the HepG2 and its subclones were arrested in the S phase in 12 h following the ethaselen treatment, and then more or less recovered in another 12 h. Meanwhile, the underlying proteins of p53, NF- $\kappa$ B and TrxR2 were partially restored from inhibition, especially in SM03. This cell cycle arrest and protein inhibition by ethaselen have been observed in previous experiments<sup>[1,7,8,10,13]</sup>. Considering the unparallel cell survival rate and the change of p53 and NF- $\kappa$ B protein levels, it was quite possible that ethaselen involved in other intracellular processes manifested at relative low dose level of TrxR inhibition. Whether it could be completely explained by TrxR1 (and/or TrxR2) inhibition and following ROS accumulation remains uncertain<sup>[7]</sup>. The analogue of ethaselen used in this study was helpful as a reference.

In brief, we defined the low dose of ethaselen based upon its IC<sub>50</sub> to maintain more than 90% cells viable. In addition, subclones derived from single HepG2 cell were used in the analysis of cell cycle distribution, intracellular protein level, colony formation and sphere growth. The analogues of ethaselen were also used in different assays as a reference. The HepG2 and its subclones differently responded in low dose ethaselen; one subclone (SM03) was sensitive for treatment of hours or weeks.

## 5. Conclusions

Low dose of ethaselen was defined as a concentration retaining >90% HepG2 cell survival in this study. We assessed the effects of low dose ethaselen on cell cycle, protein level, colony formation and sphere growth. HepG2 and its subclones differently responded to treatment of low dose ethaselen. SM03 was sensitive to treatment of low dose ethaselen, showing inhibited cell survival, large colony formation (diameter >500  $\mu\text{m}$ ) and sphere growth.

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## 低剂量乙烷硒啉对HepG2细胞亚株的影响不同

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**摘要:** 作为一种合成的、有抗肿瘤活性的有机硒, 乙烷硒啉可通过抑制硫氧还蛋白还原酶, 导致其生物学底物硫氧还蛋白处于氧化状态, 随后升高的细胞内活性氧引发细胞周期阻滞或触发凋亡。乙烷硒啉的低剂量作用尚未被研究。本研究中乙烷硒啉低剂量(1  $\mu\text{M}$ )定义为保持90%以上细胞存活的浓度。从单个HepG2细胞增殖而来的亚株(SM01, SM02, SM03)被用于低剂量乙烷硒啉效应检测。考察了低剂量乙烷硒啉对HepG2及其亚株的细胞周期转变、蛋白表达、集落形成、细胞球生长变化的影响。HepG2及其亚株对致死剂量的CDDP和5-FU显示了不同的敏感性。低剂量乙烷硒啉处理12 h后S期比例升高, 可在随后的12 h内部分恢复, 其细胞内蛋白p53, NF- $\kappa$ B等尚未完全恢复。低剂量乙烷硒啉未抑制较小的细胞集落(直径>100  $\mu\text{m}$ )形成, 未影响HepG2和SM01的细胞球生长。低剂量乙烷硒啉可显著抑制SM03细胞存活、较大细胞集落(直径>500  $\mu\text{m}$ )形成和细胞球生长, 尽管SM03细胞蛋白水平恢复较快。总体上, HepG2及其亚株对与化学物和低剂量乙烷硒啉的反应不同, 低剂量乙烷硒啉可抑制HepG2亚株(SM03)的存活和生长。

**关键词:** 低剂量; 有机硒; 细胞亚株; 异质性; 肿瘤细胞