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Imaging the Sigma-2 Receptor for Diagnosis and Prediction of Therapeutic Response

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1. Introduction

Sigma (σ) receptors represent a class of proteins that were initially classified as a subtype of the opiate receptors. Subsequent studies revealed that σ binding sites are a distinct class of receptors that are located in the central nervous system as well as in a variety of tissues and organs [1, 2]. Two σ binding site subtypes were distinguished based on differences in their drug-binding profiles and molecular weight. The two binding sites are known as σ_1 and σ_2 receptors. σ_1 receptors have a molecular weight of ~ 25 kDa, whereas σ_2 receptors have a molecular weight of ~ 21.5 kDa [3]. The σ_1 receptor has been cloned and displays a 30% sequence homology with the enzyme, yeast C8-C7 sterol isomerase [4, 5], but this receptor lacks C8-C7 isomerase activity. Recent studies have shown that neuroactive steroids bind with moderate affinity to σ_1 sites, and suggest that σ_1 receptors may modulate the activity of GABA and NMDA receptors in the CNS [6-8]. The σ_2 receptor has not been cloned, and most of what is known regarding the σ_2 receptor has been obtained through the use of in vitro receptor binding studies aimed at the pharmacological characterization of this receptor.

2. Characterization of the σ_2 receptor as a biomarker of the proliferative status of solid tumors

The first report suggesting that there is an overexpression of σ receptors in tumors cells was by Bem et al. in 1991[9]. In this study, σ binding in tumors was found to be greater than or equal to 2-fold higher than that of control nonmalignant tissue. Later, Vilner et al.[10] demonstrated that many murine and human tumor cells possess a high density of σ_2 receptors when grown under cell culture conditions. These studies clearly indicate that σ_2 receptors may serve as a biomarker for differentiating solid tumors from the surrounding normal tissues. The proliferative status of a solid tumor, which is defined as the ratio of proliferating (P) cells in a solid tumor to those driven into a quiescent (Q) state by nutrient deprivation and/or hypoxia (the P:Q ratio), is an important parameter in determining how to treat a tumor with either radiation or chemotherapy[11]. Tumors having a high

proliferative status (i.e., high P:Q ratio) typically respond better to hyperfractionated radiation therapy versus conventional radiation therapy. Similarly, tumors having a high P:Q ratio will respond better to cell cycle specific agents such as Ara-C and gemcitabine, whereas tumors having a lower proliferative status will respond better to non-cell cycle specific agents such as cisplatin and BCNU [11]. Also, a change in the proliferative status of a tumor during or after treatment has the potential to serve as a predictor of response and allow further tailoring of therapy.

In order to investigate the relationship between the density of σ_2 receptors and the proliferative status of tumors, Wheeler and colleagues used the mouse mammary adenocarcinoma cell line 66[12, 13] to determine if there was a difference in the density of σ_2 receptors in proliferating (66P) and quiescent (66Q) tumor cells in cell culture or in solid tumor xenografts. This group demonstrated that the density of σ_2 receptors in 66P cells was about 10 times greater than the density observed in 66Q cells (Figure 1A)[14]. The density of σ_2 receptors in the 66P cells was found to be quite high, ~900,000 copies/cell versus ~90,000 receptors/cell in the 66Q cells. This group also reported that the expression kinetics of σ_2 receptors follows the growth kinetics of the 66 cells (Figure 1B)[15]. A subsequent study in solid tumor xenografts of the same tumor cell lines demonstrated the identical P:Q ratio to that measured in the cell culture condition[16]. The agreement between the solid tumor and tissue culture data indicates that the σ_2 receptor is a receptor-based biomarker of cell proliferation in breast tumors. Thus the σ_2 receptor possesses properties similar to Ki-67, a marker of proliferation [17]. Ki-67 expression level is determined by immunohistochemistry using Ki-67 antibody. This method requires biopsy or surgical specimens of tumor. In contrast, radiotracers having a high affinity and high selectivity for σ_2 receptors have the potential to assess the proliferative status of human breast tumors using noninvasive imaging techniques such as Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT). In addition, it is likely that this approach can be extended to assess the proliferative status of other human tumors, such as head and neck, melanoma, and lung tumors, which are known to express a high density of σ_2 receptors[10]. Imaging the proliferative status with σ_2 receptors radiotracers can provide useful information regarding the prognosis and aggressiveness of tumors, and this information can be used to guide treatment of cancer in clinical practice. Rapidly proliferating tumors requires aggressive initial treatment. A reduction in the proliferative status of a tumor can serve as a predictor of the tumor's response to therapy.

3. Development of σ_2 receptor selective ligands

A number of structurally-diverse compounds have been shown to possess a high affinity to σ receptors [2]. However, most of these compounds bind selectively to σ_1 receptor or have similar affinities to both σ_1 and σ_2 receptors. One of the first σ_2 selective ligands reported was the benzomorphan-7-one analog, **CB-64D** [18]. This compound was identified as part of a structure-activity relationship (SAR) study aimed at refining the affinity of (-)-2-methyl-5-(3-hydroxyphenyl)morphan-7-one for μ versus κ opioid receptors [19]. A second series of compounds having a high affinity for σ_2 receptors are the 3-(ω -aminoalkyl)-1H-indole analogs [20, 21]. SAR studies with compounds that were originally designed to be serotonin 5-HT_{1A} agonists, resulted in the synthesis of **Lu 28-179** [22], also known as siramesine, which has a subnanomolar affinity for σ_2 receptors and a 140-fold selectivity for σ_2 versus σ_1

receptors. Other compounds that were found to have a higher affinity for σ_2 versus σ_1 receptors are: 1) the hallucinogen, ibogaine [23, 24]; 2) the mixed serotonin 5-HT₃ antagonist/5-HT₄ agonist **BIMU-1**[25]; 3) the tropane analog **SM-21**, an acetylcholine releaser that has been utilized as an antinociceptive agent [26, 27]; 4) the trishomocubane analog **ANSTO-19** [28]; and 5) the piperazine analog **PB28** [29].

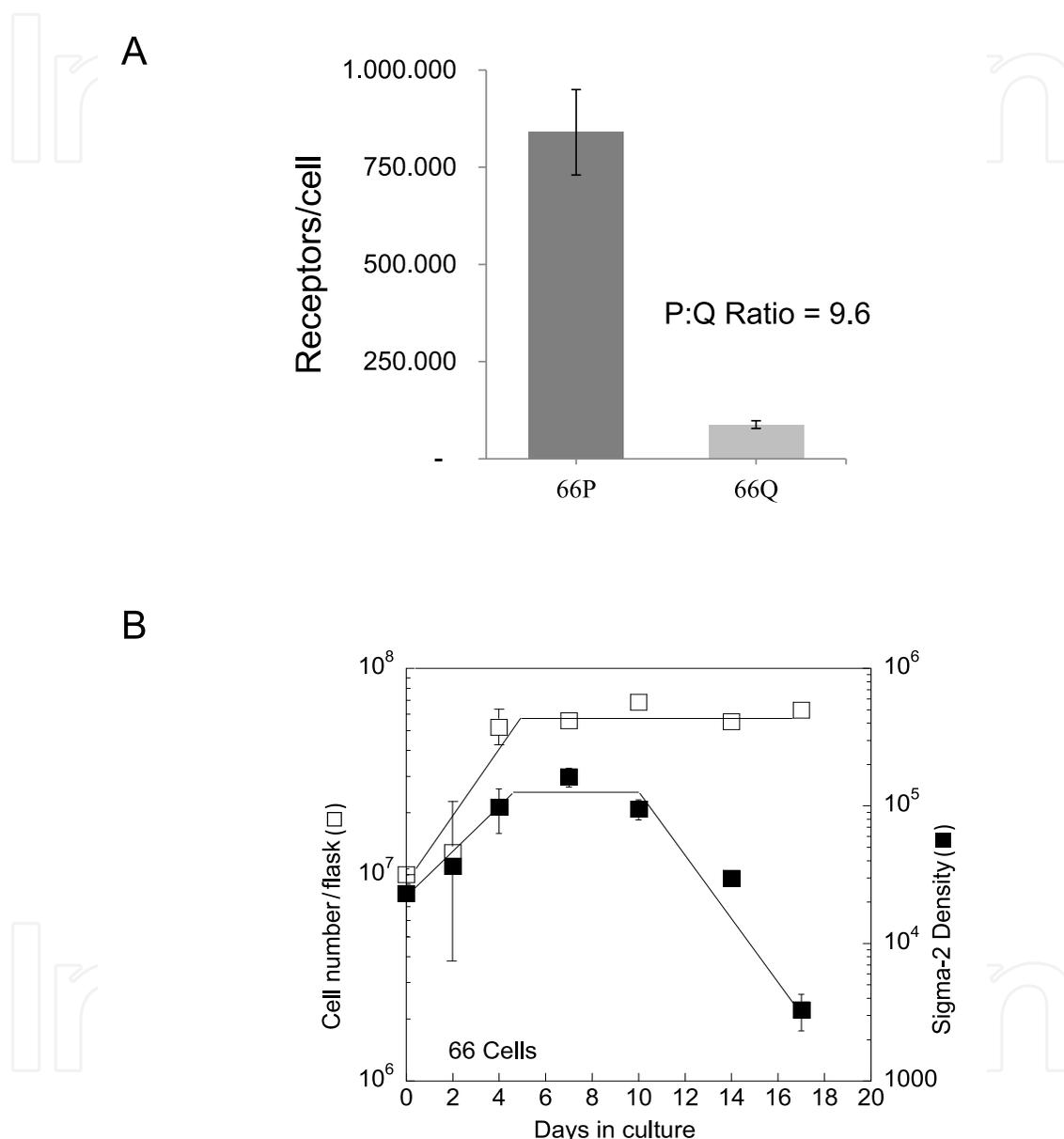


Fig. 1. The σ_2 receptor densities in proliferating and quiescent cells. A: Differences of the σ_2 receptor densities in 66P and 66Q cells. B: The σ_2 receptor expression kinetics in 66 cells during the Q to P transition and the downregulation of the σ_2 receptor densities during the P to Q transition.

A number of SAR studies using **BIMU-1** as the lead compound have identified high affinity, high selectivity σ_2 receptor ligands [30-32]. **BIMU-1** is an ideal lead compound for SAR studies since it provides a variety of regions where structural modifications can be made to

optimize the σ_2 receptor affinity and reduce the affinity for serotonin 5-HT₃ and 5-HT₄ receptors (Figure 2). The structures of **BIMU-1** were altered in three different regions: 1) replacement of the urea linkage with a conformationally-flexible carbamate moiety; 2) replacement of the N-methyl group with an N-benzyl group to diminish serotonin receptor affinity; and 3) preparation of both tropane (i.e., [3.2.1]azabicyclononane) and granatane (i.e., [3.3.1]azabicyclononane) ring systems. The most interesting analog from this initial SAR study was the compound **ABN-1**, which had a σ_2 receptor affinity of ~ 3 nM and a σ_2 : σ_1 selectivity of ~ 30 (Figure 2)[31]. Compound **ABN-1** was used as a secondary lead compound for a series of subsequent SAR studies aimed at producing second-generation granatane analogs having an improved σ_2 receptor affinity and high σ_2 : σ_1 selectivity ratio. Consequently, this led to the development of a number of fluorescent probes, **K05-138**, **SW120**, **SW107** and **SW116**, that have proven useful in two photon and confocal microscopy studies of σ_2 receptors in tumor cells growing under cell culture conditions[33, 34].

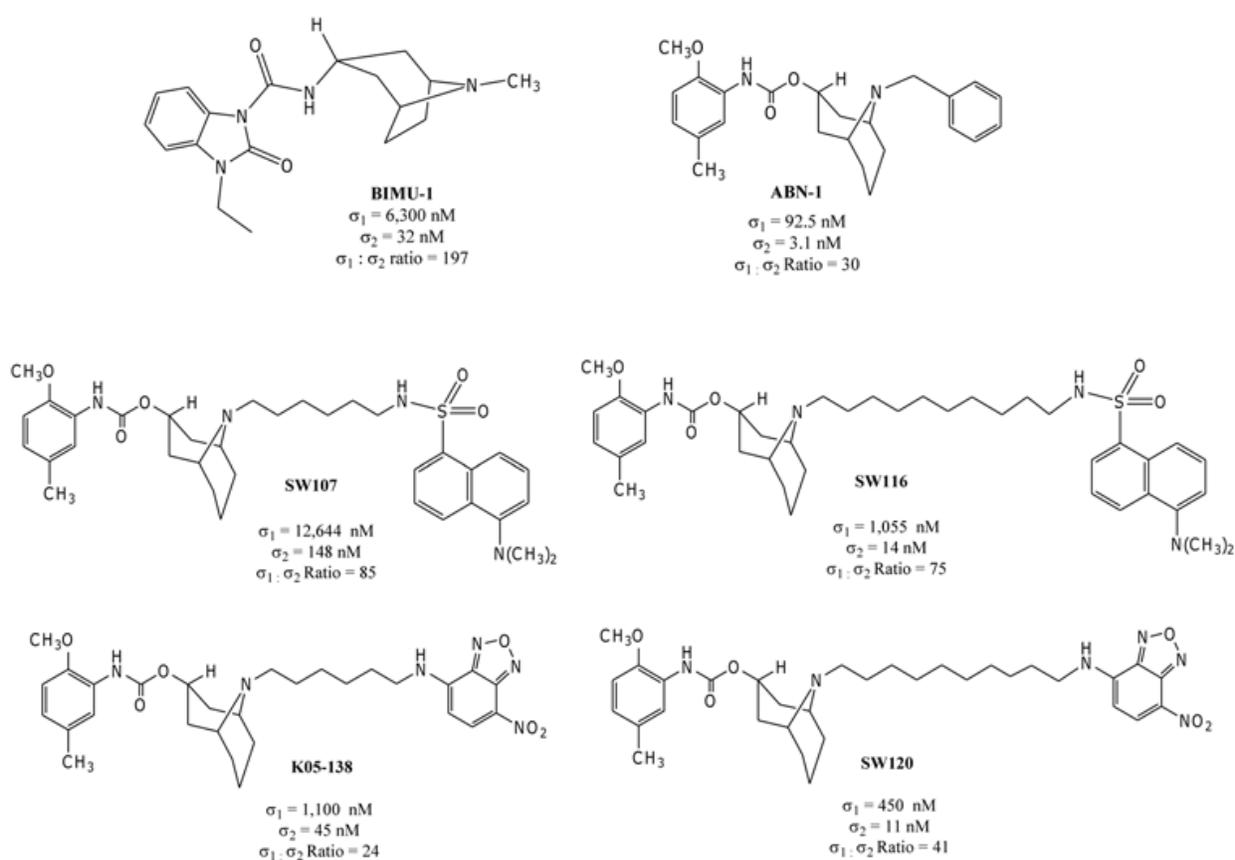


Fig. 2. Structures of **BIMU-1** and the granatane analogs. **ABN-1** was used as a lead for producing second-generation granatane-based σ_2 receptor ligands, which led to the development of a number of fluorescent probes, **K05-138**, **SW120**, **SW107** and **SW116**, for two photon and confocal microscopy studies of σ_2 receptors in tumor cells.

A second class of compounds having a high affinity for σ_2 receptors and excellent σ_1 : σ_2 selectivity ratios are the conformationally-flexible benzamide analogs. These compounds were identified in an SAR study of the benzamide analog, **YUN250**, that was developed as a dopamine D₃-selective ligand (Figure 3A) [35]. The relatively high lipophilicity of **YUN250** (log P = 5.76) suggests that it is not capable of readily crossing the blood-brain barrier and

being active in behavioral studies. In order to reduce the lipophilicity of YUN250, the 4-(2,3-dichlorophenyl) piperazine moiety of YUN250 was replaced with other aromatic amine groups. Although this strategy resulted in a number of useful dopamine D₃ receptor ligands [36], it was also observed that replacement of the 4-phenylpiperazine moiety with a 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline ring resulted in compounds having a high affinity and excellent selectivity for σ_2 versus σ_1 receptors, and a dramatic reduction in affinity for dopamine receptors [35]. As discussed in greater detail below, the conformationally-flexible benzamide analogs have proven to be an important class of σ_2 -selective compounds for the preparation of radiolabeled probes to image this receptor in vitro and in vivo.

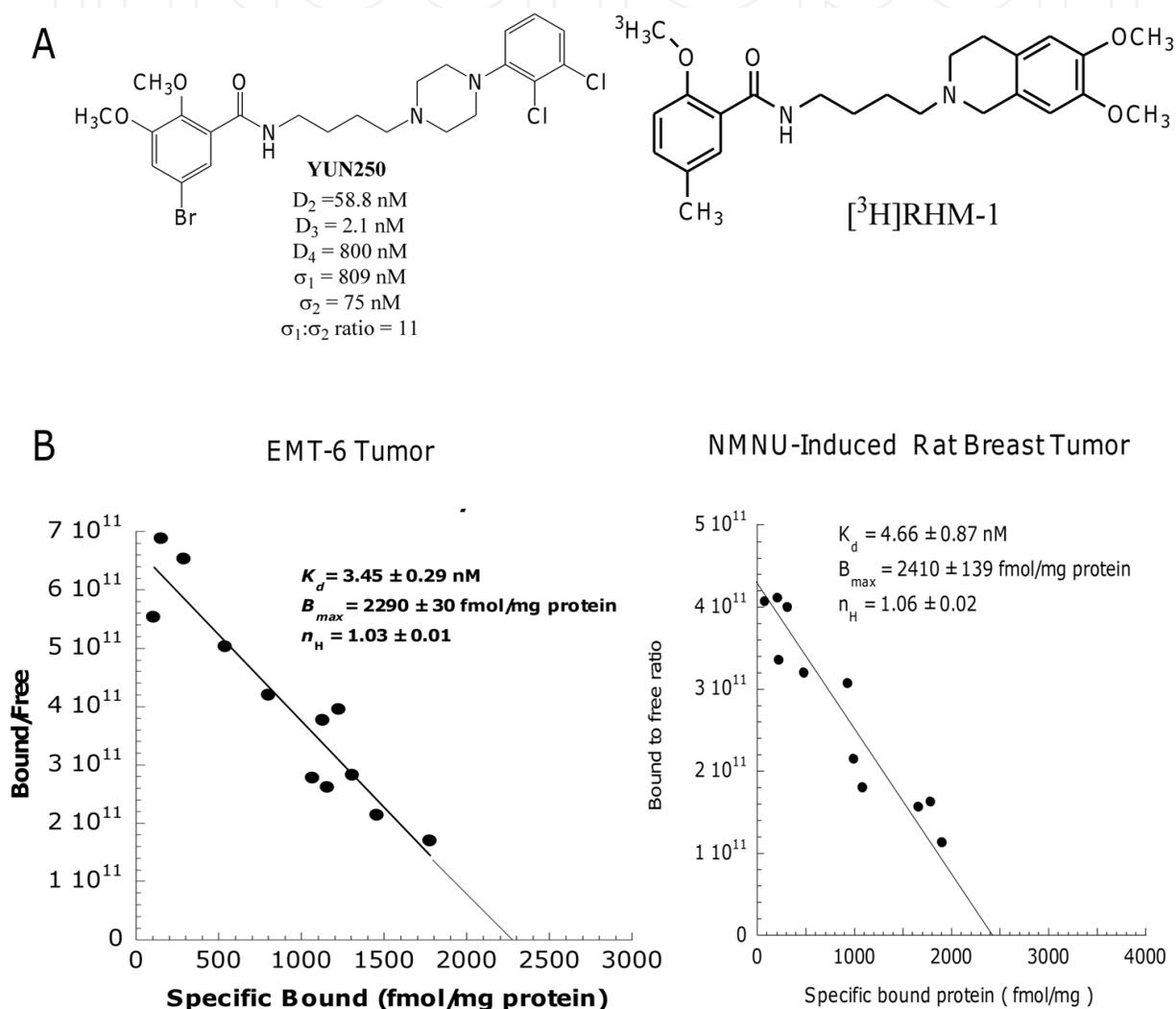


Fig. 3. The conformationally-flexible benzamide-based σ_2 receptor ligands for in vitro studies of the σ_2 receptor. **A:** YUN250 was a lead for generating the conformationally-flexible benzamide analogues which are selective for σ_2 receptors. The tritium labeled ligand, [³H]RHM-1, has been used to conduct the receptor binding assay. **B:** Scatchard studies of σ_2 receptors in EMT-6 mouse breast tumors and NMNU-induced rat breast tumors with [³H]RHM-1. This probe has high affinity to the σ_2 receptors in the rodent breast tumors and is useful for in vitro binding studies.

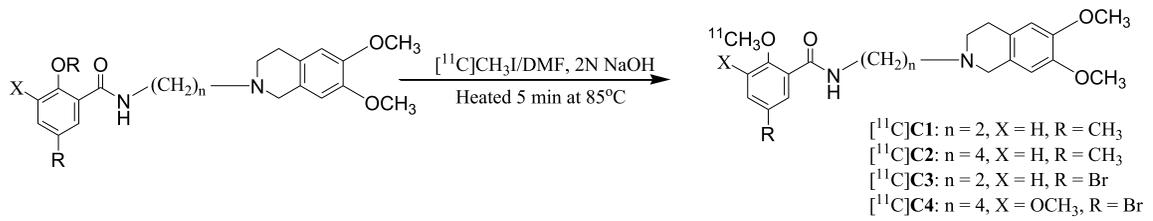
4. In vitro binding and in vivo imaging studies of σ_2 receptors

The conformationally-flexible benzamide analogs have been used to develop the σ_2 radioligands for in vitro binding studies. The tritium labeled σ_2 selective ligand, [^3H]RHM-1, has been used to conduct Scatchard studies of σ_2 receptors in tumors and normal tissues (Figure 3A)[37, 38]. The in vitro receptor binding studies indicate that [^3H]RHM-1 binds with a high affinity to both EMT-6 mouse breast tumors ($K_d=3.45$ nM) and chemically-induced rat breast tumors ($K_d=4.66$ nM). The σ_2 receptor densities of both tumors are found to be high ($B_{\text{max}}=2290$ fmol/mg of protein for EMT-6 mouse breast tumors and $B_{\text{max}}=2410$ fmol/mg of protein for *N*-methyl-*N*-nitrosourea (NMNU)-induced rat breast tumors) (Figure 3B). The pharmacologic profile of [^3H]RHM-1 is in agreement with that of [^3H]di-*o*-tolylguanidine ([^3H]DTG), a most commonly used radioligand for the σ_2 receptor binding assay. These results indicate that [^3H]RHM-1 is a useful radioligand for studying σ_2 receptors in vitro.

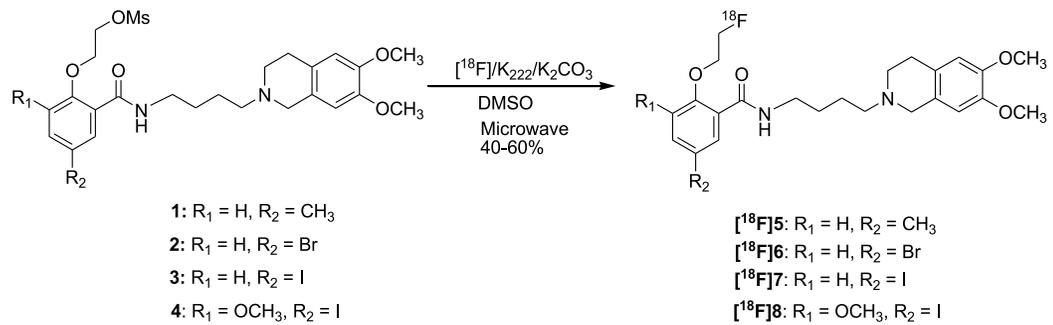
The high correlation between the density of σ_2 receptors and the proliferative status of solid tumors indicates that σ_2 selective radiotracers can be used for imaging the proliferative status of solid tumors with PET and SPECT. The conformationally-flexible benzamide analogs have been used for the development of PET radiotracers for imaging the σ_2 receptor status of solid tumors. The first PET radiotracers prepared were [^{11}C]1-4 (Figure 4A), which involves labeling the corresponding ortho-hydroxy group with [^{11}C]methyl iodide [38]. MicroPET and tumor uptake studies were conducted with [^{11}C]1-4; the most promising analog proved to be [^{11}C]4. Although all four analogs had a high affinity for σ_2 receptors, the optimal lipophilicity of [^{11}C]4 was responsible for the high tumor uptake and suitable signal: normal tissue ratios for imaging. These data indicate that both receptor affinity and lipophilicity are important properties for successful receptor-based tumor imaging agents. MicroPET and MicroCT imaging studies in a murine solid breast tumor EMT-6 clearly show the potential of [^{11}C]4 as a radiotracer for imaging the σ_2 receptor status of breast tumors with PET.

Although [^{11}C]4 provides a clear image of breast tumors in microPET imaging studies, the short half life of carbon 11 ($t_{1/2} = 20.4$ min) is not ideal for the development of radiotracers which can be used in clinical PET imaging studies. The longer half-life of ^{18}F ($t_{1/2} = 109.8$ min) compared to ^{11}C places fewer time constraints on tracer synthesis, allows imaging studies to be conducted up to 2 h after injection of the radiotracers, and usually results in higher tumor: normal tissue ratios. A number of ^{18}F -labeled radiotracers [^{18}F]5-8 (Figure 4B) based on the conformationally-flexible benzamide analogs have been generated and evaluated in murine breast tumor models[39]. The strategy involved replacement of the 2-methoxy group in the benzamide ring with a 2-fluoroethoxy group. The 2-fluoroethoxy- for methoxy-substitution is a common strategy used in the development of ^{18}F -labeled radiotracers. Biodistribution studies in female Balb/c mice bearing EMT-6 tumor allografts demonstrated that all four ^{18}F -labeled compounds had a high tumor uptake (2.5-3.7% ID/g) and acceptable tumor/normal tissue ratios at 1 and 2 h post-i.v. injection (Figure 4C). The moderate to high tumor/normal tissue ratios and the rapid clearance from the blood for [^{18}F]5 and [^{18}F]8 suggests that these radiotracers are likely the best candidates for imaging of solid tumors with PET. MicroPET imaging studies indicate that [^{18}F]5 and [^{18}F]8 are suitable probes for imaging the σ_2 receptor status of solid tumors with PET (Figure 4D). Clinical studies of [^{18}F]8 are currently in progress in the US.

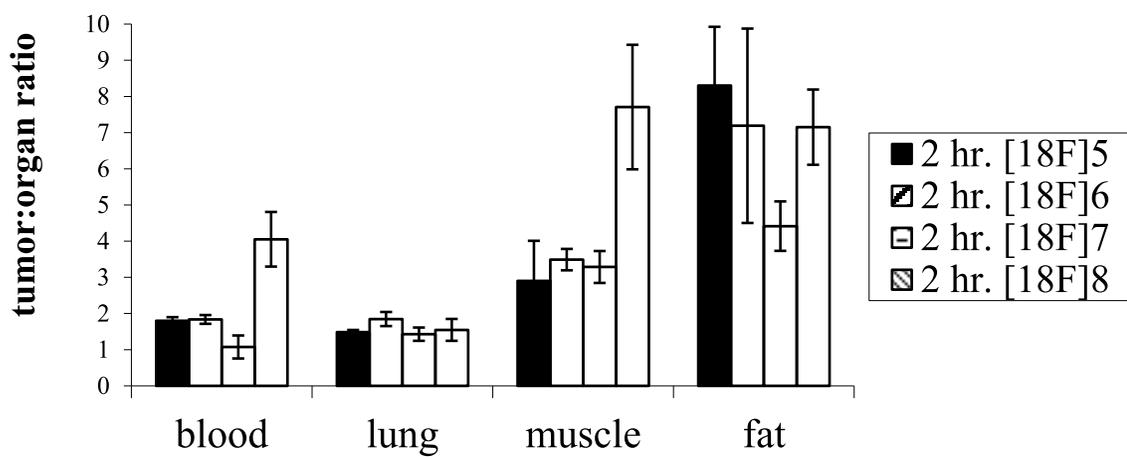
A



B



C



D

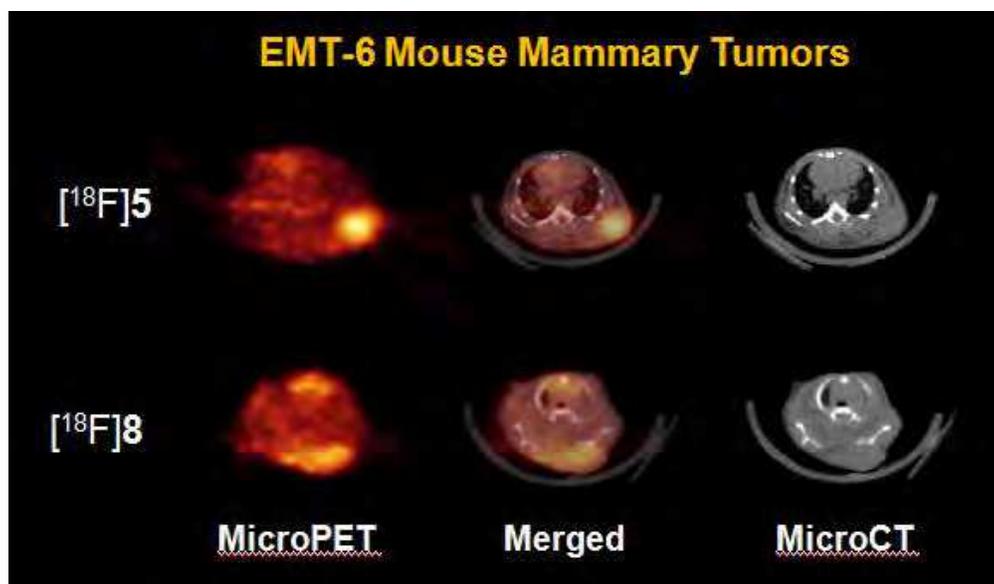


Fig. 4. The synthetic schemes for generating conformationally-flexible benzamide-based PET radiotracers. A: A number of ^{11}C -labeled radiotracers, $[^{11}\text{C}]1-4$, are prepared by labeling the corresponding ortho-hydroxy group with $[^{11}\text{C}]$ methyl iodide. B: A number of ^{18}F -labeled radiotracers, $[^{18}\text{F}]5-8$, are generated by replacing of the 2-methoxy group in the benzamide ring with a 2-fluoroethoxy group. C: Biodistribution studies in female Balb/c mice bearing EMT-6 tumor allografts demonstrating that all four ^{18}F -labeled compounds had a high tumor uptake (2.5-3.7% ID/g) and acceptable tumor/normal tissue ratios at 2 h post-i.v. injection. D: MicroPET imaging studies indicate that $[^{18}\text{F}]5$ and $[^{18}\text{F}]8$ are suitable probes for imaging the σ_2 receptor status of solid tumors with PET.

5. Confocal and two photon microscopy studies of σ_2 receptors in tumor cells

Since the σ_2 receptor protein has not been cloned, current knowledge of this receptor is based predominantly on receptor binding studies with radiolabeled probes such as $[^3\text{H}]$ DTG. The function of the σ_2 receptors has also been investigated by studying their effects on the biochemical and physiological properties of tumor cells. Several studies have shown that σ_2 ligands induced cell death. The proposed mechanisms of cell death include caspase-independent apoptosis [40], lysosomal leakage [41], Ca^{2+} release [42, 43], oxidative stress [41], ceramide production [44] and autophagy [45]. However, the subcellular localizations where the σ_2 ligands bind and function were not known. Using the fluorescent probes shown in Figure 2, Zeng and colleagues [33, 34] recently conducted a series of confocal and two photon microscopy studies that have provided the information regarding the localization of σ_2 receptors in breast tumor cells.

MDA-MB-435 cells were incubated with 30 nM **SW120** and each of the five subcellular organelle markers: the mitochondria tracker, MitoTracker Red CMXRos (20 nM), the endoplasmic reticulum tracker, ER-Tracker™ Red (500 nM), the lysosome tracker, LysoTracker Red DND-99 (50 nM), the nuclear marker, DAPI (300 nM), or the plasma membrane tracker, FM 4-64FX (5 $\mu\text{g}/\text{mL}$). The results showed that **SW120** partially co-localized with MitoTracker, ER-Tracker, LysoTracker and the plasma membrane tracker,

suggesting that σ_2 receptors may localize in mitochondria, endoplasmic reticulum, lysosomes and the plasma membrane (Figure 5). The data also showed that **SW120** did not co-localize with the nuclear marker, DAPI, suggesting that either the σ_2 receptor does not exist in the nucleus or **SW120** does not enter the nucleus. The similar results were obtained for the other σ_2 fluorescent probes (**SW116**, **K05-138** and **SW107**)[33, 34].

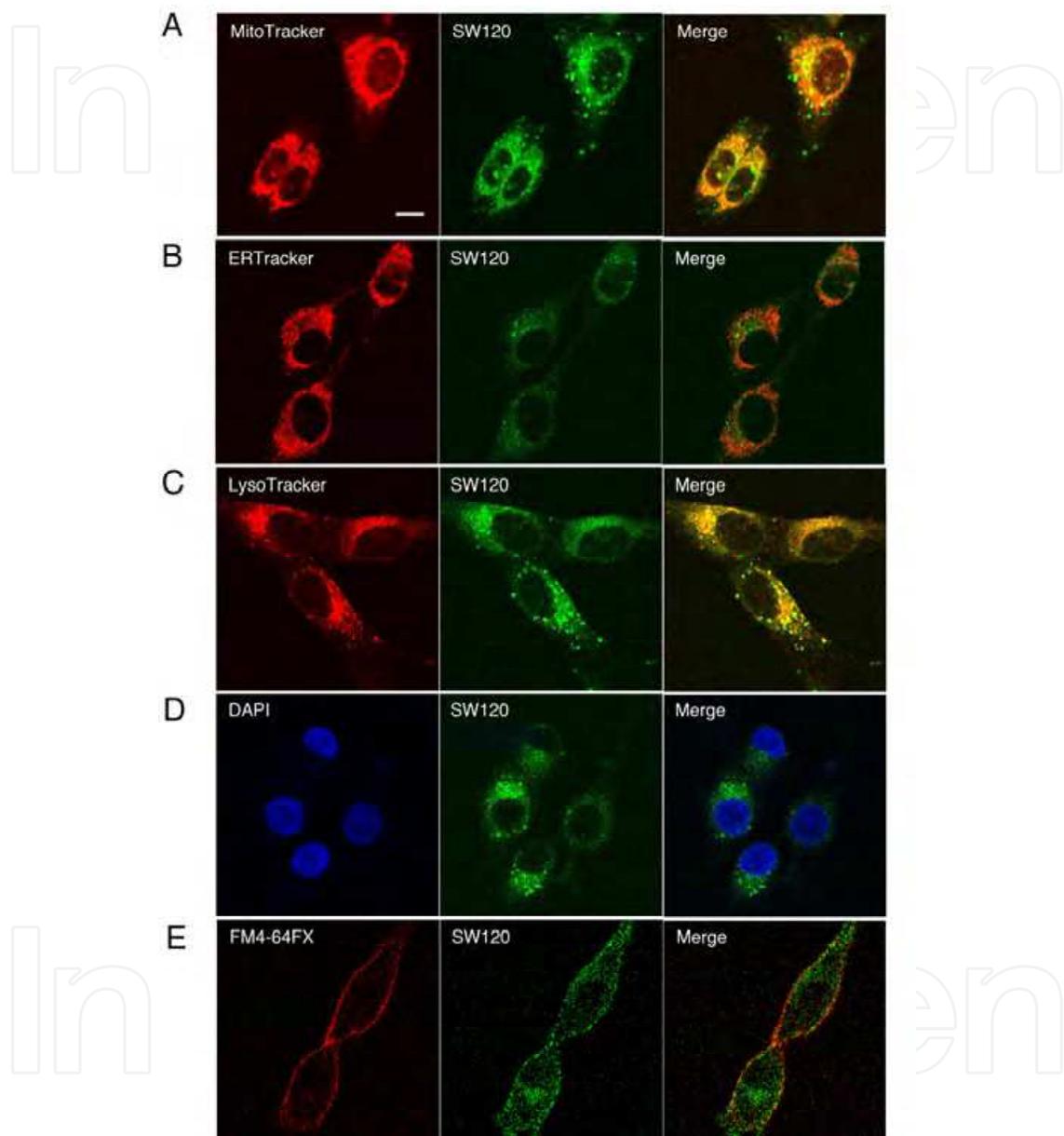


Fig. 5. Determination of the intracellular distribution of **SW120** in MDA-MB-435 cells with and without MitoTracker (A), ER-Tracker (B), LysoTracker (C), a nuclear marker, DAPI (D), or a membrane tracker, FM[®]4-64FX (E), using confocal microscopy. MDA-MB-435 cells were incubated with 30 nM **SW120** and either 20 nM MitoTracker, 500 nM ER-Tracker, 50 nM LysoTracker, or 300 nM DAPI. After incubating for 2 h at 37°C, live cells were imaged by confocal microscopy. MDA-MB-435 cells were also incubated with 50 nM **SW120** and 5 $\mu\text{g}/\text{mL}$ of the membrane tracker, FM[®]4-64FX, for 15 min at 0°C. The live cells were imaged by confocal microscopy. Scale bar = 10 μm .

The kinetic studies of internalization of σ_2 fluorescent probes in MDA-MB-435 cells were conducted using confocal microscopy [33, 34]. σ_2 fluorescent probes enter tumor cells rapidly. For example, **K05-138** and **SW120** reach 50% of the maximal fluorescent intensity ($T_{1/2}$) in 16 seconds and 11 minutes, respectively (Figure 6A). The internalization of **SW120** can be blocked by the σ_2 selective ligands, **SW43** and siramesine, by 52% and 44%, respectively (Figure 6B). This internalization can not be blocked by the σ_1 selective ligand, (+)-pentazocine. The data suggest that the internalization of the σ_2 selective ligand is partially mediated by σ_2 receptor. In order to study whether the internalization of the σ_2 ligand is mediated by endocytosis, Zeng et al examined the effect of phenylarsine oxide (PAO), a well-characterized endocytosis inhibitor [46], on the internalization of **SW120**. MDA-MB-435 cells were pretreated with 5 or 10 μM PAO for 30 min, and then treated with 10 nM **SW120** in the absence or presence of PAO for an additional 30 min. Flow cytometric analysis (Figure 6C) showed that 10 μM PAO significantly blocked internalization of **SW120** by 30%. These data demonstrate that 30% of the σ_2 receptor ligand was internalized by an endocytosis-mediated mechanism, while the remaining 70 % was internalized by other mechanisms such as passive diffusion. The rapid internalization of σ_2 receptors via endocytosis suggests that σ_2 selective ligands may potentially serve as receptor-mediated probes for delivering cytotoxic agents to solid tumors.

The two photon and confocal microscopy studies conducted by Zeng et al. [33, 34] have provided useful information for the interpretation of studies demonstrating that σ_2 receptor ligands may have a role as cancer chemotherapeutic agents. Mitochondria are a key organelle to regulate the intrinsic apoptotic pathway. Apoptotic signals such as UV irradiation or treatment with chemotherapeutic agents cause the release of cytochrome C from the mitochondria and the subsequent activation of caspase-3 leading to an apoptotic cell death [47]. The subcellular localization of σ_2 ligands in mitochondria is consistent with the previous studies that σ_2 ligands trigger apoptosis in tumor cells by acting on mitochondria [48]. The data are also consistent with our observation by transmission electron microscopy that the σ_2 ligand siramesine induces distortion of mitochondria (unpublished data). The endoplasmic reticulum (ER) serves as a dynamic Ca^{2+} storage pool [49]. σ_2 selective ligands have been reported to induce transient Ca^{2+} release from the ER, which may be responsible for σ_2 ligand-induced cell death [42]. The presence of the σ_2 fluorescent probes in the ER is consistent with these results. Additional research will be required to determine how σ_2 receptors regulate the Ca^{2+} release channels in the ER. Lysosomal proteases, such as cathepsins, calpains and granzymes, have been reported to contribute to apoptosis [50]. Under physiological conditions, these proteases are found within the lysosomes but are released into the cytoplasm upon exposure to cell damaging agents, thereby triggering a cascade of intracellular events leading to cell death. The σ_2 selective ligand siramesine has been reported to cause lysosomal leakage and induce cell death by caspase-independent mechanisms [41, 45]. The localization of fluorescent σ_2 receptor probes in the lysosomes is consistent with the hypothesis that siramesine induces cell death partially by targeting lysosomes to cause lysosomal damage, the release of proteases, and eventually cell death. Evidence has also been reported that σ_2 receptors exist in lipid rafts which are mainly found in the plasma membrane [51]. Lipid rafts play an important role in the signaling associated with a variety of cellular events including adhesion, motility, and membrane trafficking [52, 53]. The observation that σ_2 fluorescent ligands are co-localized with cytoplasmic membrane markers, and undergo receptor mediated endocytosis, is consistent with their localization in lipid rafts. How the σ_2 receptor is involved in lipid raft function deserves further investigation.

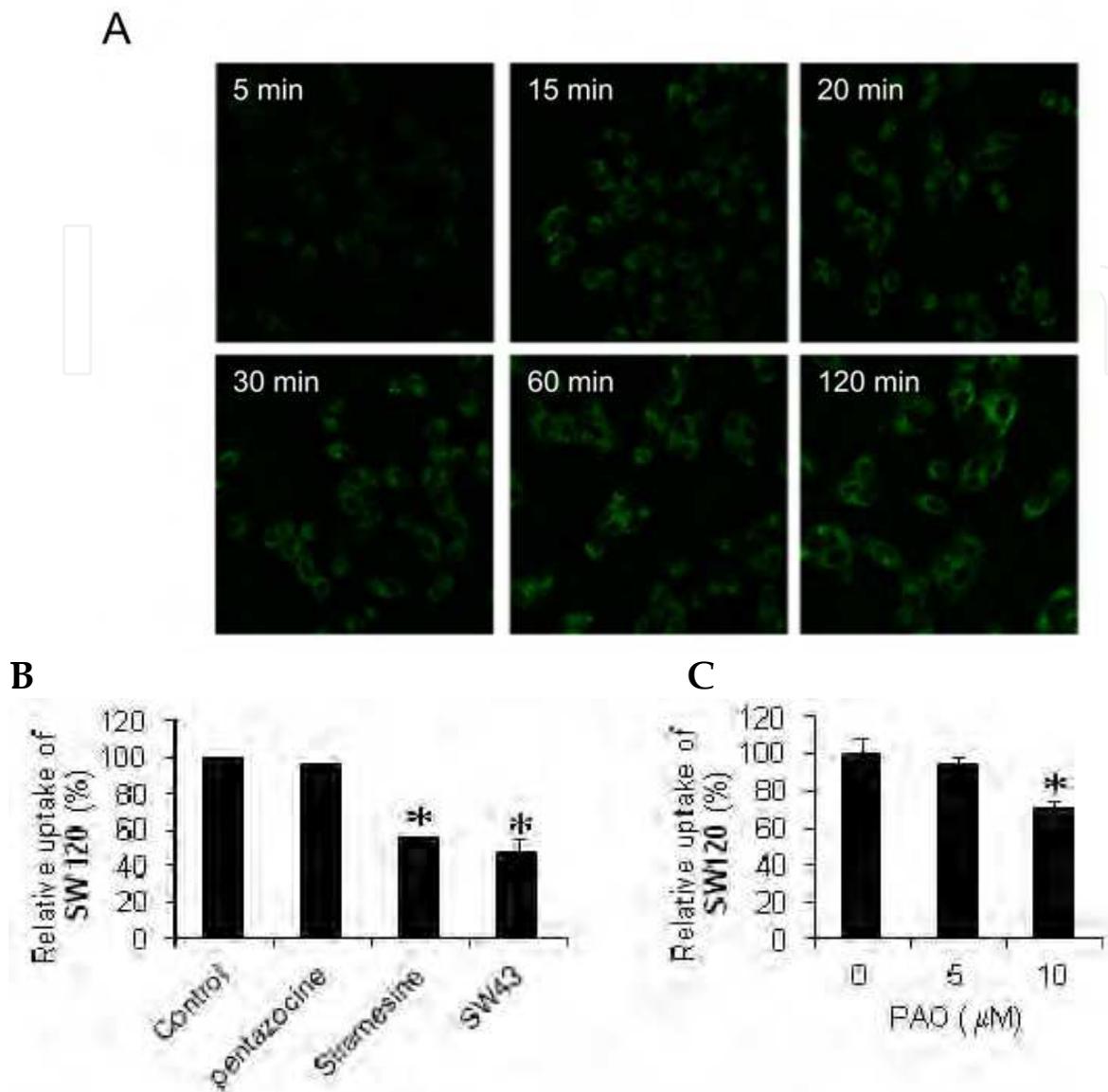


Fig. 6. Characterization of σ_2 fluorescent probes. A: The internalization kinetics of **SW120** in MDA-MB-435 cells by confocal microscopy studies show that **SW120** enters tumor cells rapidly. B: The internalization of **SW120** in MDA-MB-435 cells are partially blocked by σ_2 selective ligands, **SW43** and siramesine, but not by a σ_1 selective ligand, (+)-pentazocine. C: The inhibition of **SW120** internalization by phenylarsine oxide (PAO). MDA-MB-435 cells were preincubated with 5 or 10 μM PAO for 30 min at 37°C, and then incubated with 10 nM **SW120** for an additional 30 min. The cells were analyzed by flow cytometry. The internalization of **SW120** was significantly reduced by 10 μM PAO (* $P < 0.05$).

The σ_2 receptor has been validated as a proliferation marker in cell culture and in solid tumors. Therefore it is possible that σ_2 fluorescent probes could preferentially label proliferating cells versus non-proliferating cells and serve as agents to image cell proliferation in vivo. This hypothesis was tested in nude mice implanted with murine mammary adenocarcinoma 66 cells and BALB/C mice implanted with mouse mammary carcinoma cell line EMT6[34]. The mice were treated with **SW120** (50 μg in 100 μL PBS) for one hour. The peripheral blood mononuclear cells (PBMC), which are commonly used as

controls for non-proliferative cells, and tumor cells were prepared. These cells were analyzed by flow cytometry for **SW120** uptake and for Ki67 expression, a commonly-used proliferation marker. Our data showed that PBMC were Ki67 negative, whereas a large portion of the tumor cells were Ki-67 positive (Figure 7). The data also showed that PBMC were not labeled by **SW120**, whereas a portion of the tumor cells were labeled with **SW120**. The trend of the positive correlation between Ki67 expression and **SW120** labeling implies that the fluorescent may possess in vivo selectivity toward proliferating cells versus non-proliferative cells. These data suggest that σ_2 fluorescent probes could be used as imaging agents for monitoring cell proliferation in mice. The data also suggest that σ_2 selective ligands hold a potential to serve as therapeutic agents to selectively target tumor cells in vivo.

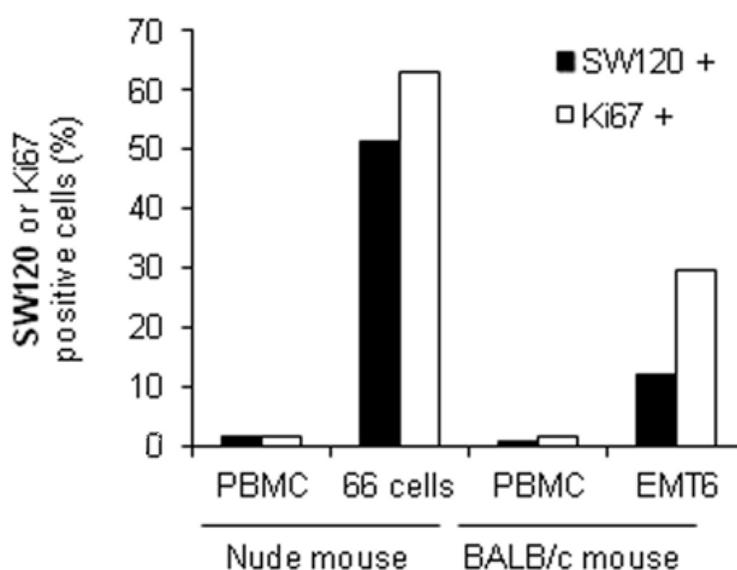


Fig. 7. Ki67 expression and **SW120** fluorescent intensity in solid mouse breast tumors and peripheral blood mononuclear cells (PBMC) of mice by flow cytometric analysis. The female nude mice were implanted with murine mammary adenocarcinoma 66 cells. BALB/C mice were implanted with mouse mammary carcinoma cell line EMT6. The mice were i.v. injected with **SW120** (50 μg / mouse) and tissues harvested after 1 hour. The 66 cells and EMT6 cells were dissociated from the solid tumors. PBMC were prepared from the blood of mice. The cells were analyzed for the fluorescent intensity of **SW120** and the Ki67 expression determined by Ki67 immunostaining using a flow cytometer. The data indicate the trend of the positive correlation between Ki67 expression and **SW120** labeling.

6. Conclusions

The σ_2 receptor continues to be an important molecular target in the field of tumor biology. The high expression of this receptor in proliferating versus quiescent breast tumors indicates that the σ_2 receptor is an important clinical biomarker for determining the proliferative status of solid tumors using the functional imaging techniques PET and SPECT. The σ_2 receptor fluorescent probes are useful to study the subcellular localization and the function of σ_2 receptors using confocal and two photon microscopy techniques, and may be used to image tumor proliferation in mice using optical imaging techniques. The full utility of the σ_2

receptor in the diagnosis and prediction of therapeutic response will rely on the cloning of the σ_2 receptor and elucidation of its functional role in normal and tumor cell biology.

Note: While this article is accepted, a paper from our group is published in Nature Communication[54]. In this paper we have identified progesterone receptor membrane component 1(PGRMC1) as the putative σ_2 receptor. This finding will greatly facilitate investigations of the functions of the σ_2 receptor in normal and tumor cells.

7. Acknowledgement

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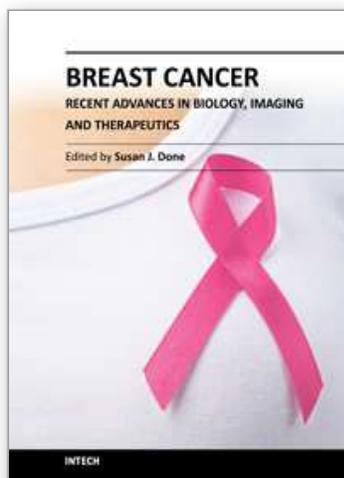
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In recent years it has become clear that breast cancer is not a single disease but rather that the term encompasses a number of molecularly distinct tumors arising from the epithelial cells of the breast. There is an urgent need to better understand these distinct subtypes and develop tailored diagnostic approaches and treatments appropriate to each. This book considers breast cancer from many novel and exciting perspectives. New insights into the basic biology of breast cancer are discussed together with high throughput approaches to molecular profiling. Innovative strategies for diagnosis and imaging are presented as well as emerging perspectives on breast cancer treatment. Each of the topics in this volume is addressed by respected experts in their fields and it is hoped that readers will be stimulated and challenged by the contents.

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