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TITLE: Small Molecules that Suppress IGF-Activated Prostate Cancers

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### Title and Subtitle
Small Molecules that Suppress IGF-Activated Prostate Cancers

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### Abstract
Elevated serum levels of insulin-like growth factor 1 (IGF1) have been found in prostate cancer patients, and IGF1-related signal transduction is thought to be an important factor in the development of prostate cancers. The goals of this project are to discover small organic molecules that suppress IGF-activated prostate cancers by cell-based screening and to analyze their action mechanisms. In the first year of funding, we discovered, from our collection of synthetic compounds, the drug-like compound we call 125B11 that suppress IGF1-dependent growth of prostate cancer cells but not serum-dependent growth. During this period of funding, we analyzed the mechanism of action of 125B11 to gain molecular insights into how IGF1 stimulates the growth of prostate cancer cells. DNA microarray and cell biological experiments indicated that 125B11 modulates the function of sterol regulatory element binding protein (SREBP), a transcription factor that activates specific genes involved in cholesterol synthesis, endocytosis of low density lipoproteins, and the synthesis of both saturated and unsaturated fatty acids. Chemical genetics of 125B11 may reveal a novel crosstalk between fatty acid metabolism and prostate cancer progression.
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Introduction

Elevated serum levels of insulin-like growth factor 1 (IGF1) have been found in prostate cancer patients, and IGF1-related signal transduction is thought to be an important factor in the development of prostate cancers (1). The goals of this project are to discover small organic molecules that suppress IGF-activated prostate cancers by cell-based screening and to analyze their action mechanisms. To discover such molecules from a chemical library, we took a unique two-step approach: we first examined the phenotypic effects of chemical library members (10,000 divergent drug-like compounds) on the insulin-induced adipogenic differentiation of cultured fibroblasts, and then identified, from the pool of the non-cytotoxic compounds that blocked the insulin-induced adipogenesis, organic compounds that suppress IGF-mediated growth of prostate cancer cells. In the first year of funding, we successfully discovered 125B11 through the two-step approach. The chemical genetic analysis of 125B11 may reveal new insights into how IGF1 stimulates the growth of malignant prostate cancer cells.

Body

In the second year of funding, we focused on Task 2: To analyze mechanism of action of 125B11

In the first year of funding, we discovered 125B11, a compound that suppress IGF1-dependent growth of prostate cancer cells. The drug-like thiazole derivative specifically inhibited the IGF1-induced growth of DU-145 cells at IC50 of 0.1 μM but had little effects on their serum-induced growth (Fig. 1) (2). IGF1-induced phosphorylation of Akt and MAPK in DU-145 cells was unaffected by 125B11, suggesting that 125B11 inhibits the cell-proliferative function of IGF1 in a way independent of the known IGF1-signaling pathway. In the second year of funding, we focused on analyzing the mechanism of action of 125B11.

![Fig. 1 Discovery of 125B11. 125B11 inhibited the IGF1-induced growth but not the serum-induced growth. DU-145 cells were treated with varied amounts of 125B11 in the presence of IGF1 or 2% fetal bovine serum (FBS).](image-url)
Microarray experiments of 125B11
Gene expression profiling comparison in the drug-treated and -untreated cells by DNA microarray technology might reveal specific molecular pathways affected by the drugs. DU145 prostate cancer cells were treated with 125B11 or DMSO alone in the presence of IGF1, and extracted mRNA was analyzed by Affimetrix DNA microarrays at the Microarray Facility in Baylor College of Medicine. To our surprise, we found that the genes downregulated by 125B11 included those known or likely to be controlled by sterol regulatory element binding protein (SREBP), a transcription factor that activates specific genes involved in cholesterol synthesis, endocytosis of low density lipoproteins, and the synthesis of both saturated and unsaturated fatty acids (3). These include LDL receptor and HMG-CoA reductase, a target of cholesterol-lowering drug statins. We hypothesized that 125B11, whether directly or indirectly, inhibits SREBP or its pathway.

Table 1
Microarray analysis of 125B11

<table>
<thead>
<tr>
<th>Genes known to be controlled by SREBP</th>
<th>Genes relevant to sterol/fat synthesis</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Accession</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDLR</td>
<td>NM_004243</td>
<td>Low density lipoprotein receptor</td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>NM_004265</td>
<td>HMG-CoA reductase</td>
</tr>
<tr>
<td>SREBP</td>
<td>NM_004265</td>
<td>Sterol regulatory element binding protein</td>
</tr>
<tr>
<td>iA-2</td>
<td>NM_004265</td>
<td>Insulin-induced protein</td>
</tr>
<tr>
<td>iB-1</td>
<td>NM_004265</td>
<td>Insulin-induced protein</td>
</tr>
<tr>
<td>iC-3</td>
<td>NM_004265</td>
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</tr>
<tr>
<td>iD-4</td>
<td>NM_004265</td>
<td>Insulin-induced protein</td>
</tr>
</tbody>
</table>

DAMD17-03-1-0228 PI: Uesugi, M.
125B11 blocks cleavage of SREBP in cells

Goldstein, Brown, and colleagues have demonstrated that in the presence of sterol, 125 kDa SREBP is located endoplasmic reticulum membranes. When cellular sterol concentrations were lowered, the amino terminal 68 kDa domain of SREBP is released from the membrane, entered the nucleus, and bound to SREs in the promoters of SREBP-target genes. In contrast, the cleavage of SREBPs was prevented when cells were loaded with cholesterol/oxysterols; the result was low nuclear levels of SREBP and low rates of transcription of SREBP target genes (Fig. 2) (4).

We examined if the cleavage of SREBP is blocked by 125B11 in cells. Western blot analysis showed that treatment with 5 μM of 125B11 increased the amounts of uncleaved 125Kda SREBP (Fig. 3).

It remains unknown how 125B11 inhibits the cleavage of SREBP. In sterol-deprived cells, a protein called SCAP functions as a chaperone protein and transports the 125 kDa SREBPs from the endoplasmic reticulum to the Golgi where two proteases (S1P and S2P) cleave SREBP to generate 68Kda SREBP (Fig. 2) (3). In contrast, in cholesterol-loaded cells, SCAP and SREBP fail to migrate to the Golgi and the 125 kDa SREBP remains in ER. Thus, under these conditions, maturation and nuclear localization of SREBPs are reduced. 125B11 may modulate one of these transport/cleavage process. Experiments are planned for the next year.
SREBP plays a role in IGF1-dependent growth of prostate cancers?

Our results suggest that 125B11 suppress IGF1-dependent growth of prostate cancer cells by blocking SREBP activation. If this hypothesis is correct, then siRNA knockdown of SREBP suppress IGF1-dependent growth of prostate cancers. We stably transfected siRNA expression vector of SREBP to DU145 cells and examined its growth in the presence of IGF1. Although we need more validation experiments, the knockdown cells seem to insensitive to IGF1. This result may suggest that SREBP plays an essential role in IGF1-dependent growth and prostate cancer progression. Our continued experiments may reveal an interesting crosstalk between cholesterol metabolism and prostate cancers.

Key research accomplishments

- Identified SREBP-responsive genes as genes downregulated by 125B11
- Obtained evidence suggesting that 125B11 blocks the cleavage of SREBP
- Obtained preliminary results suggesting that SREBP plays an essential role in IGF1-dependent growth of prostate cancers.

Reportable outcomes

None during this funding period.
Results were discussed in 2004 Prostate Cancer Foundation Retreat

Conclusion

There is no change in Tasks and experimental design. As originally planned, discovery of 125B11 in the first year of funding enabled its mechanistic analysis in the second year. Our analysis indicated the potential role of SREBP in IGF1-dependent growth of prostate cancers. Continued experiments may reveal a molecular understanding of the relationship between cholesterol metabolism and prostate cancer progression. We are also interested in understanding how 125B11 blocks the cleavage of SREBP. Such studies may lead to the discovery of a new drug target.
References


Appendix

None