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TITLE: Targeting ID to Inhibit the Vascularization of Breast Tumors

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CONTRACTING ORGANIZATION: Sloan-Kettering Institute for Cancer Research
New York, New York 10021

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13. ABSTRACT *(Maximum 200 Words)*

Under the auspices of this Army Concept Award we have begun the derivatization of a compound (AIL, for anti-Id lead) shown to bind to the Id proteins, known to be required for the formation of blood vessels into breast tumors. We have identified sites on AIL that can be modified and have begun the chemistry of adding a linker to this region of the molecule that can accept geldanamycin. The AIL-geldanamycin fusion will be used to target the Id proteins for degradation in human cancers and thereby inhibit tumor angiogenesis.

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Table of Contents

Introduction:

The Id proteins have recently been identified by my laboratory as being essential for the growth and maturation of blood vessels in murine breast tumor xenografts (1). Even partial loss of Id function leads to a profound reduction in the vascularization of tumors with a concomitant loss of growth and metastatic potential. These observations, the fact that Id proteins are expressed at very low levels in adult mice and humans and the fact that the precise mechanism of Id action has been defined make these proteins extremely attractive targets for anti-angiogenic drug design in the treatment of human cancers. The research being proposed here focuses on the novel derivatization of a lead compound which we have shown interacts with Id and inhibits its activity in vitro and in cell culture models. (called AIL for anti-Id lead) . The derivative compounds will be tested for their efficacy in the inhibition of the vasculature of breast cancer xenografts and genetically induced breast cancers in mice.

Thus, our recent observation that the Id proteins are required for the vascularization of breast cancer xenografts directs our search for novel compounds that can inhibit Id and thereby the growth and metastasis of breast tumors. The detailed understanding we have of the mechanism of action of Id will facilitate the identification of compounds that exhibit high specificity and low toxicity and therefore are likely to be important clinical tools in the treatment of this disease.

Body:

In order to derivatize the AIL-lead compound with geldanamycin, we first needed to determine which sites on the AIL could be modified without affecting its ability to interact with Id proteins. Five AIL-derivatives with different sites modified were tested for their ability to compete with 3H-AIL as a measure of relative affinity. As shown in figure 1, several derivatives competed for 3H-AIL as well as unlabelled native AIL whereas others did not. Based on the chemical structure of these derivatives we have now begun to focus our attention on the fusion of geldanamycin to linkers attached to sites on AIL that do not affect its activity. Thus far the linkers fused to AIL have proven to be yield chemically unstable derivatives (by spectroscopic measurements) but modification of those linkers is currently underway.

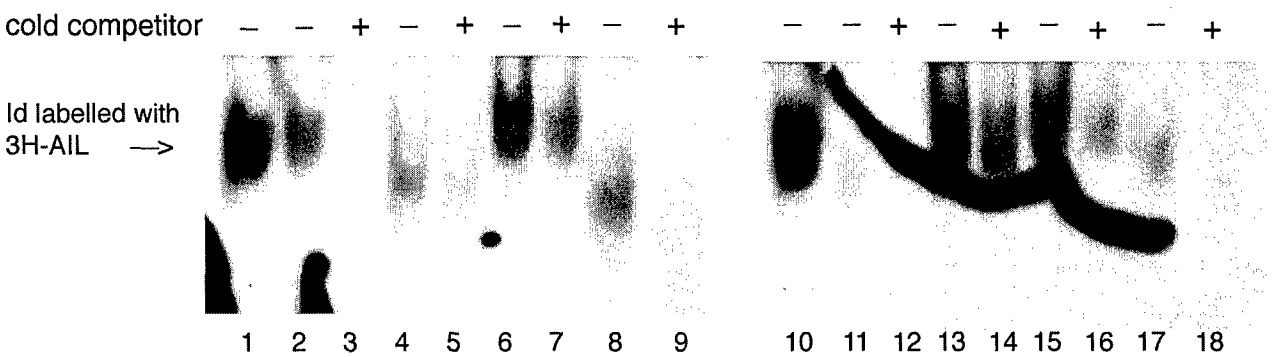

Figure 1. Competition assay to determine the ability of cold AIL derivatives to inhibit the binding of 3H-AIL to Id1. 3H-AIL was incubated with purified Id protein and the mixture analyzed by native gel electrophoresis. 3H-AIL bound to Id1 is visualized by autoradiography in the position indicated with the arrow. 3H-AIL alone does not enter the gel (not shown). Unlabelled derivatives of AIL are tested for their ability to inhibit the binding of 3H-AIL to Id1. Lanes 1,10: 3H-AIL with no competitor. Lanes 2,3: carrier for derivative 1 and 100 fold excess derivative 1, respectively. Lanes 4,5 and 6,7 and 8,9 and 17,18: carrier (lanes 4,6,8) and derivatives 2,3,4 and 5 (lanes . Lanes 11,12 and 13,14 and 15,16: carrier (lanes 11,13,15) plus 3 concentrations of cold AIL (lanes 12>14>16) .

Further efforts should continue to produce more stable AIL-linker derivatives that will allow the fusion of AIL to geldanamycin and testing for its ability to inhibit Id activity.

Key Research Accomplishments:

• Development of a competition assay for the binding of AIL-derivatives to Id using 3H-AIL

• Identification of sites on the AIL suitable for linker attachment and derivatization

Reportable Outcomes:

This work has led to a more thorough development of an anti-Id strategy for the treatment of breast cancer. The ideas and results generated have help me to secure a $225,000 award from the Breast Cancer Research Foundation to continue this work.

Conclusions:

The development of an assay for the ability of AIL derivative to bind Id is an important step forward in the identification of compounds that can inhibit Id activity. This assay has facilitated the identification of sites on the AIL that will not perturb its ability to bind Id and should allow us to place linkers on the AIL for the purpose of fusion to geldanamycin. The linkers tested thus far have not produced stable compounds. Other linkers must now be tested and once stable compounds generated, fusion to geldanamycin can be performed and tested for maintenance of their ability to bind and inhibit Id. This may lead compounds capable of inhibiting blood vessel formation around breast cancers.

DEPARTMENT OF THE ARMY
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