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Re-evaluation of inhibin α subunit as a tumour suppressor in prostate cancer

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Abstract

Inhibin is a member of the TGF-β superfamily of growth and differentiation factors and a tumor suppressor. Consistent with the tumor suppressive function of the inhibin α subunit in prostate cancer, we reported a loss of gene expression due to DNA hypermethylation and loss of heterozygosity (LOH) as well as down regulation of inhibin α subunit immunoreactivity. Paradoxically, an expanded study to evaluate the prognostic significance of inhibin α subunit expression in men with prostate cancer resulted in a contradictory outcome, whereby an up-regulation of subunit expression was recorded. In seeking a more comprehensive explanation for all data sets, we offer a unifying hypothesis. We propose that the tumor suppressor activities of the inhibin α subunit dominate in non-malignant tissue, but its oncogenic activities emerge during tumorigenesis. An explanation such as this, involving a switch in gene function from being tumor suppressive to pro-oncogenic, may account for the discrepant findings in other types of cancer.

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1. Introduction

Carcinogenesis is a multi-step process whereby malignant transformation occurs through a sequence of events. Beginning with premalignant changes, tumor progression results in well differentiated, poorly differentiated, and metastatic disease although there is considerable variation in the rate of progression in different organs or tissues.

In the prostate, adenocarcinomas develop slowly and the interval between the initiation of prostatic intraepithelial neoplasia (PIN) lesions and androgen independent metastatic disease can span decades. At each stage of progression there are genetic changes and an accumulation of successive mutations to oncogenes, tumor suppressor genes and genes such as those involved in apoptosis and DNA repair. At any given time there will be tumor heterogeneity related to the progressive genetic damage and the sequential appearance of different characteristics of the tumor cells such as alterations in growth, motility, invasiveness, and immune modulation. Tumor cell heterogeneity arises early in carcinogenesis and is maintained throughout tumor progression.

2. Evidence that inhibin α subunit is a tumor suppressor

Classification of inhibin α subunit as a tumor suppressor resulted from the observation that inhibin α null mice develop gonadal or adrenal tumors after castration (Matzuk et al., 1992). Similarly, in the human prostate, inhibin α is considered to play a tumor suppressive role. Inhibin α subunit protein and mRNA levels are low or undetectable in a number of prostate cancer cell lines including LNCaP, PC3, and DU145 (van Schaik et al., 2000; Batres et al., 1995; Fürst et al., 1995; Ying et al., 1995). In the benign human prostate, inhibin α subunit mRNA and protein is expressed (Thomas et al., 1998; Mellor et al., 1998) but down regulated in malignant human prostate tissue (Mellor et al., 1998). In an independent set of patient samples, we demonstrated that the mechanisms of DNA hypermethylation and loss of heterozygosity (LOH) contribute to loss of inhibin α subunit expression (Schmait et al., 2002). In addition, the inhibin α subunit promoter is methylated, and thus inactivated, in the human prostate cancer cell lines, LNCaP, DU145, and
PC3 (Balanathan et al., 2004). Both DNA methylation and LOH are common mechanisms of inactivating tumor suppressor genes in malignancy. Therefore, a number of independent studies using human tissue and cell lines support the concept that inhibin α subunit is a tumor suppressor in the prostate and is switched off in the progression to malignancy.

3. Evidence that contradicts the premise that inhibin α subunit is a tumor suppressor

Less than 50% of men who undergo radical prostatectomy to treat prostate cancer are cured of their disease. A retrospective study was undertaken in order to further investigate and compare the difference in inhibin α subunit expression in non-malignant and malignant regions of tissue specimens from men with prostate cancer. Our aim was to determine if the tumor expression of inhibin α subunit correlated with the failure to cure men with Gleason grade 4/5 cancer after radical prostatectomy.

A collection of specimens was available from men who had undergone radical prostatectomy at Stanford Medical Center from 1983 to 1992. Comprehensive histological reviews of the specimens were available together with an estimate of the percentage of the total cancer represented by Gleason grade 4 or 5. Prior analysis of the cases had demonstrated that the % Gleason grade 4/5 was the strongest predictor of the failure to be cured by surgery (Stamey et al., 1999). Our investigations were confined to a group of

![Image of immunostaining in biopsy specimens](image-url)

Fig. 1. Immunostaining in biopsy specimens from men with prostate cancer in regions of non-malignant (a, c, e) or malignant (b, d, f) tissue. Panels a and b show the failure to detect immunoreactive inhibin α subunit using the Groome R1 antibody. Panels c and d show inhibin α subunit immunoreactivity is localized to the epithelial cells of non-malignant regions of the specimen, whereas no staining was observed in corresponding tumor regions using the polyclonal antibody n; a result consistent with the previous report of down regulation or loss of inhibin α subunit immunoreactivity as described by Mellor et al. (1999). Panels e and f show the up-regulation of inhibin α subunit immunoreactivity in malignant compared to non-malignant regions of tissue using the monoclonal antibody, PO412.
difficulties arising mainly from a single group of investigators required a rigorous re-evaluation of the data. The discrepancies between the results of the Stanford study and our previous studies (that mainly utilize needle biopsy specimens) might have been due to several factors. The earlier studies using needle biopsy rather than resected specimens included fewer patients than the 107 used at Stanford and may have reflected a sampling bias in the earlier studies. In all of the studies antigen retrieval techniques were required to detect inhibin α subunit staining and differences in fixation techniques either between institutions, or in the fixation properties of needle biopsy versus prostatectomy specimens, might have contributed to variation. An important difference relates to the antibodies used in all of these studies. The most commonly used inhibin α subunit antibody is the Groome R1 antibody raised to the amino acids 3-24 of the inhibin α subunit (Robertson et al., 2001). However, this antibody fails to detect inhibin α subunit immunoreactivity in the prostate (Fig. 1a and b). This observation was observed between Monash and Stanford and agrees with a report in which inhibin A was not detected in seminal fluid (Anderson et al., 1998). In seeking a more comprehensive explanation for all these data, we offer a unifying hypothesis (Fig. 2).

Our earlier studies utilized tissue specimens from men with various grades of prostate cancer. Prostate cancer is a multi-step process involving the initial transition from non-malignant to malignant status and, following pre-malignant lesions and localized prostate cancer, metastasis and alteration in hormonal responsiveness predominate. In the non-malignant state the activities of tumor suppressors dominate, including the inhibin α subunit. We postulate that the loss of the inhibin α subunit in low grade prostate cancer reflects a loss of a tumor suppressor and predict that other tumor suppressor activities decline. However, as disease progression continues, we postulate oncogenic activities prevail and there is a switch in function and expression of the inhibin α subunit that becomes oncogenic and pro-metastatic. Up-regulation of inhibin α subunit would indicate a more advanced disease status and metastasis. Therefore in our paradigm (Fig. 2), the tumor suppressor activities of the inhibin α subunit would dominate in non-malignant tissue but during tumorigenesis its oncogenic and pro-metastatic activities would emerge.

An explanation such as this, involving a switch in gene function from being tumor suppressive to pro-oncogenic, may account for the discrepant findings in ovarian carcinoma (Matzuk et al., 1992; Robertson et al., 1999). As well, inhibin is a member of the TGF-β superfamily and future studies will need to demonstrate that inhibin or the inhibin α subunit, like TGF-β, has dual positive and negative effects on tumorigenesis (Wakefield and Roberts, 2002). Whether or not this hypothesis prevails in other types of cancer warrants further investigation.

4. Resolution of the paradox

The slow but steady accumulation of discrepant findings leads to confusion. In attempting to explain the internal

Table 1

<table>
<thead>
<tr>
<th>Centers</th>
<th>Total number of patient biopsies</th>
<th>Number of patient biopsies showing up-regulation of inhibin α subunit</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>18 (62%)</td>
</tr>
<tr>
<td>B</td>
<td>32</td>
<td>18 (56%)</td>
</tr>
<tr>
<td>C</td>
<td>21</td>
<td>14 (66%)</td>
</tr>
<tr>
<td>D</td>
<td>22</td>
<td>5 (23%)</td>
</tr>
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</table>

Biopsy specimens from men with prostate cancer were selected at each of four Australian centers and using the protocols developed by Monash and Stanford Universities to detect immunostaining in radical prostatectomy specimens, the level of immunostaining for inhibin α subunit was compared for each patient. At each center a subset of patients were identified in which inhibin α subunit immunoreactivity was up-regulated ranging from 23 to 66%.

174 men with 72% grade 4/5 cancer because these men had an intermediate rate of failure, providing the opportunity to analyze the prognostic potential of inhibin α subunit as a marker of disease progression.

The results of the study were unexpected and did not support the previous evidence that inhibin α subunit was down regulated in men with prostate cancer (Fig. 1c and d). The majority of cases (99%) showed more, rather than less, intense staining of inhibin α subunit in cancer compared to the non-malignant tissue (Fig. 1e and f). Those men whose tumor tissue had the more elevated expression of inhibin α subunit had a higher risk of recurrence, although this association was not statistically significant. The study concluded that the inhibin α subunit was frequently over-expressed in high-grade prostate cancer.

The discrepancy in the results of the Stanford study compared to previous studies by the same investigators was unexpected. However, in four independent laboratories in Australia further studies were undertaken to sample inhibin α subunit staining in biopsy specimens from men with high-grade prostate cancer. At each center, groups of patients were selected to compare inhibin α subunit immunostaining in malignant versus non-malignant regions of the same tissue specimen. The results showed that inhibin α subunit immunoreactivity was up-regulated in 23–66% of patients.

Interestingly, these data highlighted a previous discrepancy in the molecular analysis of methylation, in which we reported a subset of patient specimens that did not display inactivation of the inhibin α promoter region (Schmitt et al., 2002). The corresponding pathology reports on these patients identified cribriform carcinoma that is commonly associated with poor patient outcome.

Appendix
Fig. 2. In order to resolve the apparent paradox related to inhibin α subunit in men with prostate cancer we propose the following paradigm represented in this schematic diagram. In non-malignant tissues the activities of tumor suppressors dominate but decline as premalignant and malignant progression occurs resulting in a change in the balance between tumor suppressors and oncogenes so that eventually oncogenic activities dominate in the tumor. As well we propose that there is a switch in the activity of the inhibin α subunit gene that is initially tumor suppressive but latterly oncogenic and pro-metastatic. We postulate that this switch, known to occur with TGF-β is surprisingly also a feature of the inhibin α subunit.

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References


