Extracellular Catalytic Subunit Activity of the cAMP-dependent Protein Kinase in Prostate Cancer

Mary Ellen Cvijic, Tsunekazu Kita, Weichung Shih, Robert S. DiPaola, and Khew-Voon Chin


ABSTRACT

The role of cAMP in cell growth and differentiation, gene expression, and neuronal function is mediated by the cAMP-dependent protein kinase (PKA). Differential expression of type I and type II PKA has been correlated with neoplastic transformation and differentiation, respectively. PKA is primarily an intracellular enzyme. However, it has been demonstrated that PKA may be associated with the plasma membrane and is exposed to the extracellular environment. Here we report the first evidence for the presence of a free extracellular kinase activity of PKA in the growth media of cultured prostate and other cancer cells, as well as in plasma samples from prostate cancer patients. This PKA activity is specific due to its phosphorylation of the PKA-specific substrate kemptide and its inhibition by the potent and specific PKA inhibitor PKI, but not by other protein kinase-inhibitory peptides. Intriguingly, this exoprotein kinase activity is cAMP independent, suggesting that only the catalytic subunit is secreted, and therefore the kinase activity is not modulated by the regulatory subunit of PKA. Western blot analysis of the culture supernatant from prostate cancer cells indicates the presence of the catalytic subunit. This increase in extracellular PKA catalytic subunit activity in prostate cancer may have profound effects on the tumorigenesis of prostate cancer and may serve as a novel marker and therapeutic target for the disease.

INTRODUCTION

Phosphorylation mediated by the cAMP signal transduction pathway can be elicited by various physiological ligands and is critically involved in the regulation of metabolism, cell growth and differentiation, and gene expression (1–4). The PKA3 holoenzyme is composed of two genetically distinct subunits, the catalytic (C) and regulatory (R) subunits, forming a tetrameric holoenzyme R2C2, which in the presence of cAMP dissociates into R4(cAMP)4 dimer and two free catalytically active catalytic subunits.

For approximately 40 years, the regulatory subunit has been the only known receptor for cAMP in cells, and cAMP binding to the holoenzyme has been the accepted mechanism that regulates PKA activity. This dogma of cAMP signaling is being rewritten to accommodate some recent discoveries that implicate the existence of alternative mechanisms for the cAMP messenger system. The first hint of a novel alternative mechanism for cAMP signaling came from studies that showed the direct interaction of cAMP with some ion channels in the central nervous system (5–7). This was followed by a study that demonstrated that the catalytic subunit can be activated in a cAMP-independent subunit-independent manner in a ternary complex of NFxB-IxB-catalytic subunit (8). Degradation of IxB after exposure to inducers of NFxB leads to activation of the catalytic subunit in a cAMP-independent manner and subsequent phosphorylation of NFxB. In another series of studies, using PKA genetic mutants of CHO cells, it was shown that the regulatory subunit may have functions independent of the catalytic subunit kinase activity in influencing cellular sensitivity to chemotherapeutic agents (9, 10). Furthermore, the cytochrome c oxidase subunit Vb was shown to interact with the RIIa subunit and is regulated in a cAMP-dependent manner. This interaction alters the cytochrome c oxidase activity and the release of cytochrome c from the mitochondria (11). Most recently, a novel family of cAMP-binding guanine nucleotide exchange factors was identified that can selectively activate the Ras superfamily of guanine nucleotide-binding protein Rap1 in a cAMP-dependent but PKA-independent manner (12, 13). Although the cAMP signaling mechanism is one of the best understood biochemical pathways, these alternative mechanisms of cAMP- and PKA-mediated signaling demonstrate the versatility of the pathway in regulating cellular processes.

The presence of PKA activity in the extracellular surface of cells is another intriguing phenomenon that is not very well understood (14, 15). Studies on protein phosphorylation activity in extracellular domains did not lag far behind the discovery of intracellular protein kinases. Ectoenzymes are defined as membrane-bound enzymes whose catalytic activities are localized on the extracellular cell surface, whereas exoenzymes are enzymes whose activities are present in the extracellular environment with-

Received 11/30/99; revised 3/8/00; accepted 3/8/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by National Cancer Institute Grants CA67722 (to K-V. C.), CA77135, and CA80654 (to R. S. D.).

2 To whom requests for reprints should be addressed, at The Cancer Institute of New Jersey, 195 Little Albany Street, New Brunswick, NJ 08901. Phone: (732) 235-6196; Fax: (732) 235-7493; E-mail: Chinkv@umdnj.edu.

3 The abbreviations used are: PKA, cAMP-dependent protein kinase; NFxB, nuclear factor kb; exo-PKA, PKA exoprotein kinase; ecto-PKA, PKA ectoprotein kinase; PKC, protein kinase C; PSA, prostate-specific antigen; LDH, lactate dehydrogenase; CHO, Chinese hamster ovary.
out being directly associated with cells and can be isolated as soluble proteins (16). Various ecto- and exoenzymes are implicated in developmental processes, intercellular communication, feedback regulation, and events such as cell-cell interaction or receptor transduction of external stimuli.

Several studies suggest that PKA, a predominantly intracellular enzyme, may also be an ectoprotein kinase associated with the cell surface. PKA activity has been found at the surface of glioma cells and on the outer surface of rat spermatozoa and rat adipocytes (17–19). PKA was also detected as an exoenzyme in rabbit serum after thrombin stimulation of platelets (20). It has been shown that phosphorylation of the PKA-specific substrate kemptide occurs at the surface of HeLa cells (21, 22). The atrial natriuretic peptide has also been shown to be phosphorylated in a variety of intact cells by a cAMP-dependent ectoprotein kinase (23). More recently, it has been definitively shown that purified membrane of human LS-174T colon carcinoma cells contained PKA activity (24). The ecto-PKA can be photoaffinity-labeled with 8-azido-[32P]cAMP, and the regulatory subunits of the holoenzyme can be immunoprecipitated with specific antibodies (24). In addition, both intracellular cAMP and ATP have been shown to be released into the extracellular space and are present in body fluids, thus providing the factors necessary to influence PKA activity extracellularly and therefore supporting a potential role for PKA in intercellular communication and regulation (16, 21, 23).

To evaluate the relevance of exo-PKA activity in cancer, we determined the status of exo-PKA activity in the growth media of some cancer cell lines. We found significant exo-PKA activity in prostate cancer cells and a few other cancer cell lines. Studies were extended to include plasma samples from prostate cancer patients, and our results revealed elevated extracellular PKA activity in 50% of the patient samples. These results suggest that exo-PKA may play a role in prostate tumorigenesis and may have significant clinical implications as a novel molecular marker as well as a target of therapeutics in prostate cancer.

**MATERIALS AND METHODS**

**Cell Culture.** CHO cells (10001, 10248, 10260, 10215, and 10265) were cultured in α-MEM containing 10% fetal bovine serum. The mouse adrenocortical carcinoma cells Y1 and Kin 8 were grown in α-MEM with 15% heat-inactivated horse serum and 2.5% heat-inactivated newborn calf serum. Cell lines derived from cancers of the prostate (LnCAP and PC3), breast (MCF-7 and MDA-MB-231), lung (H1299 and A549), ovary (A2780 and CP70), cervix (C33A and SiHa), and colon (HT29) were cultured in RPMI 1640 with 10% fetal bovine serum. The melanoma (MNT-1), liver (PLC/PRF/5), and epidermoid (A431) cancer cell lines were cultured in DMEM containing 10% fetal bovine serum. All media were supplemented with 2 mM glutamine, 50 units/ml penicillin, and 50 μg of streptomycin, and the cells were maintained in a humidified atmosphere of 95% air and 5% CO2 at 37°C.

**PKA Assay.** To determine exo-PKA activity in the growth media of cell lines, approximately 1 × 10^6 cells/well were plated in 96-well dishes in 100 μl of tissue culture media containing heat-inactivated serum and then cultured overnight. On the following day, 90 μl of media were collected from the wells and spun at low speed to remove detached cells. Exo-PKA activity in the media was determined by measuring the transfer of 32P from [γ-32P]ATP to kemptide (Sigma Chemical Co., St. Louis, MO), a specific substrate for PKA. The reaction mixture contained 27.5 mM 4-morpholinepropanesulfonic acid; 2-(N-morpholino)ethanesulfonic acid, 1.0 mM EDTA, 2.75 mM NaF, 5.5 mM magnesium acetate, 1.25 mg/ml BSA, 0.1 mg of kemptide, 100 μM cAMP, and 5.0 μCi of [32P]ATP in a total volume of 160 μl. Some of the reactions contained either 10 μM PKI (Life Technologies, Inc.), a specific PKA inhibitor, or 10 μM protein kinase C inhibitor peptide (Life Technologies, Inc.). The reaction was initiated by the addition of media supernatant. After 10 min of incubation at 30°C, 40-μl aliquots were immediately spotted onto phosphocellulose discs (Whatman P81 filter paper), washed four times in 3% phosphoric acid, and quantitated by scintillation counting. A no-enzyme blank was subtracted from the total incorporation. We have arbitrarily set a predetermined level of at least >1000 cpm in the level of 32P incorporation into the substrate kemptide for the sample to be considered as harboring significant levels of PKA catalytic subunit activity.

**Plasma Preparation and Exo-PKA Assay with Patient Samples.** Plasma was obtained from patients (baseline samples before chemotherapy) who had histologically proven prostate cancer and initial local therapy (radical prostatectomy or definitive radiation therapy) and were enrolled on a prior clinical study (25). In patients with prior prostatectomy, PSA was >2 ng/ml. In patients with prior radiation therapy, PSA was >7 ng/ml, with a rate of increase (PSA velocity) of >0.4 ng/ml/month. Patients had Karnofsky performance of >70% and adequate blood counts, liver function, and renal function. All patients were required to give written informed consent, which was approved by the Institutional Review Board at the Robert Wood Johnson Medical School. Plasma from normal healthy individuals was also included as a negative control. Ninety-μl aliquots were used for the exo-PKA activity assay as described above. The nonparametric Wilcoxon rank-sum test was used to assess the difference in the exo-PKA levels between the normal samples and prostate cancer samples.

**Western Blot Analysis.** For Western blot analysis of PKA expression, 1.5 × 10^6 cells were cultured in 60-mm Petri dishes. Approximately 12 h after plating, cells were washed with PBS, replenished with 1 ml of serum-free media, and incubated for an additional 24 h. Media were collected and centrifuged to remove detached cells, and the volume was concentrated by freeze-drying to approximately 60 μl. The samples were separated by 10% SDS-PAGE, transferred to polyvinylidene difluoride membranes, and incubated overnight with a 1:2000 dilution of a polyclonal antibody against the catalytic subunit of PKA (a gift of Dr. Michael Gottesman; NIH, Bethesda, MD). The immunoreactive bands were detected with an ECL kit (Pierce Chemical) using a secondary goat antimouse antibody conjugated to horseradish peroxidase (Life Technologies, Inc.).

**LDH Assay for Determination of Cell Lysis.** For quantitative determination of LDH in serum, the Paramax LDH reagent, which employs the lactate to pyruvate conversion at alkaline pH, was used to evaluate cell lysis. The change in
absorbance as a result of the reduction of NAD\(^+\) to NADH is measured according to the manufacturer’s specifications. The normal LDH range determined from a population of normal fasting individuals is 94–172 units/liter. LDH activity was also determined in cell culture media.

RESULTS

The association of ecto-PKA activity with the plasma membrane in various cells and tissues is well documented. However, the presence of exo-PKA, that is, activity present extracellularly without direct association with cells and that can be isolated as soluble proteins, is less well understood. In this study, we evaluated the presence of exo-PKA in the tissue culture media of a series of cancer cell lines. Exo-PKA assays were conducted using kemptide as a specific substrate as described in “Materials and Methods.” Media containing heat-inactivated serum were used because fetal bovine serum contained PKA-specific enzyme activity (data not shown) that was not present in the heat-inactivated media (Fig. 1). Furthermore, it has been reported previously that fetal bovine serum contains some protein kinase activities (26).

We also examined the exo-PKA activity in other prostate cancer cell lines and found significantly elevated levels of kinase activity in most of the prostate cell lines with the exception of Du145 (Fig. 2B). The exo-kinase activity is specific for kemptide and was inhibited by PKI but not by the PKC peptide inhibitor, suggesting that free catalytic subunit is secreted out of the cells. We conducted Western blot analysis to further determine the presence of catalytic subunit in serum-free culture media from these prostate cells, using an anticalyptic subunit antiserum. Results in Fig. 3 showed the presence of catalytic subunit in the culture media of the prostate cancer cell line LnCAP and PC3. This was consistent with the results obtained from the exo-kinase activity assays. In contrast, the mouse adrenocortical carcinoma cell line Y1 has a low level of exo-PKA activity, but its PKA mutant derivative, Kin 8, exhibited a high level of exo-PKA activity, which is consistent with the defective PKA in this cell line (27). No exo-PKA activity was detected in the wild-type CHO cell line (10001) and its various PKA mutant derivatives (10248, 10260, and 10215; Fig. 1).

We also examined the exo-PKA activity in other prostate cancer cell lines and found significantly elevated levels of kinase activity in most of the prostate cell lines with the exception of Du145 (Fig. 2B). The exo-kinase activity is specific for kemptide and was inhibited by PKI but not by the PKC peptide inhibitor, suggesting that free catalytic subunit is secreted out of the cells. We conducted Western blot analysis to further determine the presence of catalytic subunit in serum-free culture media from these prostate cells, using an anticalyptic subunit antiserum. Results in Fig. 3 showed the presence of catalytic subunit in the culture media of the prostate cancer cell line LnCAP and PC3. This was consistent with the results obtained from the exo-kinase activity assays. In contrast, the mouse adrenocortical carcinoma cell line Y1 has a low level of exo-PKA activity, but its PKA mutant derivative, Kin 8, exhibited a high level of exo-PKA activity, which is consistent with the defective PKA in this cell line (27). No exo-PKA activity was detected in the wild-type CHO cell line (10001) and its various PKA mutant derivatives (10248, 10260, and 10215; Fig. 1).
subunit in the media of the cultured prostate cells. We did not observe the presence of catalytic subunit in Du145 cells (Lane 5), consistent with the low levels or lack of exo-PKA activity in these cells (Fig. 2B).

To demonstrate the clinical relevance of exo-PKA or the extracellular secretion of the catalytic subunit of PKA, plasma samples from 14 prostate cancer patients were examined for exo-PKA activity. Our results showed striking levels of exo-PKA activity in seven patients or 50% of the samples evaluated (Fig. 4A). Plasma samples from seven normal individuals (three females and four males) showed negligible levels of exo-PKA activity. This difference in exo-PKA activity between the prostate cancer and normal samples was statistically significant ($P = 0.04$). The secretion of exo-PKA was independent of the PSA...
levels and histological grade of the tumor (Table 1). Further analysis demonstrated that the exo-kinase activity from the plasma samples of normal or prostate cancer individuals was specific for kemptide, was inhibited by PKI but not by the PKC peptide inhibitor, and was not stimulated by cAMP (Fig. 4B).

Because the exo-PKA activity, which could catalyze the labeling of kemptide, in the media or plasma samples could have been released from lysed cells during the preparative procedure, we then determined the LDH activity to evaluate potential cell lysis. As shown in Fig. 5, LDH activity of the plasma samples from prostate cancer patients were within the normal range (94 – 172 units/liter) and were not significantly different from the normal control, thus excluding the possibility that the exo-PKA activity might be a result of cell lysis. Evaluation of the culture media of the prostate cancer cell lines also yielded negligible LDH activity (data not shown).

DISCUSSION

In this study, we have presented the first evidence of the presence of exo-PKA catalytic subunit activity in the growth media of various cultured cancer cell lines including some prostate cancer cells, as well as in the plasma samples of prostate cancer patients. This exo-kinase activity is specific for kemptide, a PKA-specific substrate, and is inhibited by PKI but not by the PKC peptide inhibitor. Western blot analysis shows the presence of catalytic subunit in the growth media of prostate cancer cell lines. Furthermore, the LDH assays demonstrate that exo-PKA activity is not likely a result of contaminating PKA activity from lysed cells during sample preparation. Our results suggest that this exo-kinase activity is the catalytic activity of PKA, therefore implicating that the catalytic subunit of PKA may be secreted extracellularly in human malignancies.

The functional significance of the presence of exo-PKA catalytic subunit in cancer is unclear. However, studies from ecto-protein kinases may shed light on the functions of the exo-PKA activity observed in our studies. The physiological functions for ecto-protein kinases seem to be cell-type specific. For example, in T lymphocytes, an ecto-protein kinase phosphorylates proteins involved in T-cell-mediated cytotoxicity (29). In basophils, phosphorylation of membrane proteins by an ectoprotein kinase involves transmembrane influx of Ca\(^{2+}\) (30). Exo-PKC is found to be involved in the homeostasis of platelets, maintaining their resting state (31). Other ectoprotein kinases play important roles in early events of platelet activation and secretion (32). Furthermore, extracellular kinases are implicated in the phosphorylation of β-amyloid peptides and may function in the etiology of neurodegenerative disorders such as Alzheimer’s disease (33). In U937 cells, an ectoprotein kinase may regulate cell interactions and immune responses (34). In Swiss 3T3 cells, an ectoprotein kinase is involved in the activation of a cell growth inhibitor (35). These studies and many others have all demonstrated the presence of ecto-protein kinase activities on the extracellular surface and their effects on various physiological processes. However, demonstration of the presence of exo-PKA, which is a freely soluble PKA activity, has not been documented.

What is the physiological function of this secreted PKA catalytic subunit in the plasma? It has been shown that overexpression of the PKA Ca\(^{2+}\) subunit decreases the adherence of cells to vitronectin, laminin, and collagen I and enables cells to migrate through these extracellular matrix components (29). PKA did not regulate adhesion to or migration through fibronectin and did not appear to be associated with changes in expression of surface integrins. In addition to modulating tumor adhesion and migration in vitro, PKA activation caused an increased formation of me-
tastases from s.c. tumors but did not regulate the formation of experimental metastases by i.v. injected tumor cells. These results suggest that PKA signaling is important for modulating the tumor-extracellular matrix interaction and can facilitate tumor transit from the primary tumor site (36). Although this study did not address whether there are exo-PKA activities in the culture media, we speculate that some of the excess Cα subunit produced may be secreted or transported out of the cells as a soluble kinase in the extracellular milieu and may contribute to tumor progression and metastasis.

Fig. 4 PKA activity in the serum of prostate cancer patients. A, patient plasma samples (ES18, GS13, JC02, RL06, HL10, GF29, NS24, HG21, LS22, RS08, CM16, SA12, EK17, and EO33) were evaluated for PKA activity and compared with negative control volunteers (GD, TP, and VHV, female controls; TK, WY, KO, and VPV, male controls). B, specificity of PKA kinase activity in patient plasma samples. Ninety μl of patient plasma sample (HL10) and normal female (VHV) and male (VPV) controls were used to assay for exoprotein kinase activity. Certain samples contained 100 μM cAMP, whereas others contained 10 μM PKI, the PKA-specific inhibitor, or 10 μM inhibitory peptide for PKC. All values are normalized to a no-enzyme blank.
It has also been shown that 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine H-7 (a PKA and PKC inhibitor) and \( n \)-(2-[methylamino]ethyl)-5-isoquinoline-sulfonamide H-8 (a cAMP- and cGMP-dependent protein kinase inhibitor) significantly reduced the number of lung metastases in 3LL (Lewis lung carcinoma) tumor cells (37). These results suggest that the protein kinase inhibitors could inhibit the formation of lung metastases and that PKA may play a role in promoting tumor growth and metastasis. The compounds H-7 and H-8 inhibit the catalytic subunit kinase activity by competitively inhibiting

Table 1  Patient data and PKA activity compared with healthy individuals

<table>
<thead>
<tr>
<th>Patients</th>
<th>PSA (ng/ml)</th>
<th>Age (yrs)</th>
<th>Radiation therapy</th>
<th>Prostatectomy</th>
<th>Current use of androgen ablation therapy</th>
<th>Gleason score</th>
<th>Exo-PKA activity (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS18</td>
<td>147</td>
<td>66</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>7/10</td>
<td>534</td>
</tr>
<tr>
<td>GS13</td>
<td>269.7</td>
<td>66</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>7/10</td>
<td>24</td>
</tr>
<tr>
<td>JCO2</td>
<td>61</td>
<td>70</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>5/10</td>
<td>982</td>
</tr>
<tr>
<td>RL06</td>
<td>3.9</td>
<td>55</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>6/10</td>
<td>348</td>
</tr>
<tr>
<td>HL10</td>
<td>10.1</td>
<td>69</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>7/10</td>
<td>9705</td>
</tr>
<tr>
<td>GF29</td>
<td>3.1</td>
<td>66</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>8/10</td>
<td>4079</td>
</tr>
<tr>
<td>NS24</td>
<td>41</td>
<td>81</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>NA</td>
<td>957</td>
</tr>
<tr>
<td>HG21</td>
<td>5.4</td>
<td>67</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>7/10</td>
<td>2535</td>
</tr>
<tr>
<td>LS22</td>
<td>7.6</td>
<td>68</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>7/10</td>
<td>297</td>
</tr>
<tr>
<td>RS08</td>
<td>19.5</td>
<td>70</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>7/10</td>
<td>2355</td>
</tr>
<tr>
<td>CM16</td>
<td>11.1</td>
<td>65</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>7/10</td>
<td>6445</td>
</tr>
<tr>
<td>SA12</td>
<td>51.2</td>
<td>58</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>7/10</td>
<td>8667</td>
</tr>
<tr>
<td>EK17</td>
<td>2.2</td>
<td>71</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>7/10</td>
<td>2206</td>
</tr>
<tr>
<td>EO33</td>
<td>37.1</td>
<td>80</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>7/10</td>
<td>164</td>
</tr>
<tr>
<td>GD(\text{a})</td>
<td>NA</td>
<td>52</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>210</td>
</tr>
<tr>
<td>TP(\text{a})</td>
<td>NA</td>
<td>41</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>73</td>
</tr>
<tr>
<td>VHV(\text{a})</td>
<td>NA</td>
<td>24</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>689</td>
</tr>
<tr>
<td>TK(\text{a})</td>
<td>NA</td>
<td>42</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>240</td>
</tr>
<tr>
<td>WY(\text{a})</td>
<td>NA</td>
<td>34</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>242</td>
</tr>
<tr>
<td>KI(\text{a})</td>
<td>NA</td>
<td>40</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>370</td>
</tr>
<tr>
<td>VPV(\text{a})</td>
<td>NA</td>
<td>25</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>743</td>
</tr>
</tbody>
</table>

\(\text{a}\) NA, not applicable.
\(\text{b}\) Normal healthy individuals. GD, TP, and VHV, females; TK, WY, KI, and VPV, males.

Fig. 5  LDH activity in patient plasma samples. Lactate-to-pyruvate conversion was determined by measuring the absorbance as a result of the reduction of \(\text{NAD}^+\) to \(\text{NADH}\). WL, a healthy male volunteer used as negative control.
ATP binding to the catalytic subunit. It is conceivable that the inhibition of PKA activities by H-7 and H-8 may occur both intra- and extracellularly, resulting in the inhibition of metastasis. Although we observed low levels of exo-PKA catalytic subunit activity in the culture media of two lung cancer cell lines (Fig. 1), this does not rule out the possibility that lung cancer may secrete PKA catalytic subunit into the extracellular milieu. Additional experiments need to be conducted to investigate exo-PKA activity in lung cancer cells and its potential role in tumor metastasis.

It is also important to note that both intracellular cAMP and ATP have been shown to be released into the extracellular space and are present in body fluids, thus providing the factors necessary to influence the exo- and ecto-PKA activities extracellularly (16, 21, 23). The presence of exo-PKA catalytic subunit in culture media of various cancers and in the plasma of prostate cancer patients suggests that this anomalously secreted kinase activity may mediate intercellular growth response signals or cell attachment and adhesion properties involved in the multistep process of prostate cancer progression and metastasis. One of the major hindrances of clinical treatment of prostate cancer is the difficulty in predicting the course of the disease. The high incidence of poor prognosis as a result of metastatic prostate cancer warrants further development of other markers for early-stage prostate cancer and prediction of the aggressiveness of the disease. The possibility that the activity of the exo-PKA catalytic subunit from our study may serve as an indicator of disease status in prostate cancer and other malignancies needs to be further explored.

To further understand the physiological significance and molecular mechanisms of exo-PKA function, a search for the natural substrates and the significance of their phosphorylation is also required. The fact that high levels of exo-PKA catalytic subunit activity are found in various cancer cell lines and in the plasma samples of prostate cancer patients suggests the exciting possibility that this activity may serve as a novel molecular marker for cancer. More experimentation will be needed to evaluate the effects of exo-PKA on cell growth properties and tumor progression and metastasis.

REFERENCES


