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著者の申告すべき利益相反なし

Short Communications

Usefulness and perceived-barriers of patient-held-records in palliative care settings: the OPTIM-study

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The primary aim of this study was to describe how patient-held-records was used in the regional palliative care program (OPTIM-study). The number of patient-held-records disseminated was 1,131 per region per year. A total of 15% and 16% of 706 physicians and 2,236 nurses in the region reported that they used patient-held-records disseminated during the study periods, respectively. In-depth interview identified themes [difficulty in dissemination], potential benefits of patient-held-records ([improved sense-of-control of patients], [improved information sharing among health care professionals]), and barriers ([lack of patient-perceived benefits and patient burden], [necessity that all health care professionals involved should be aware the value of patient-held-records and understand how to use it]). Of 11 hospitals who introduced patient-held-records, only 2 hospitals continued to use it during 3-year study periods. In conclusion, region-wide dissemination of patient-held-records seems to be unfeasible in many regions in Japan.
Palliat Care Res 2012; 7(2): 382-8

Key words: palliative care, region, patient-held-record

Table 1 Descriptions about patient-held-records

1. Current status
[The notebook is used by some patients, but not widely]
"Some patients thoroughly utilize the material, and others do not use it at all. Patients who attend hospital frequently do not necessarily bring it each time. A patient undergoing anticancer drug treatment keeps various documents, including the results of examinations and descriptions of drugs, bound in the notebook 'My Medical Chart'. Another patient punches holes in those documents and puts them in their original file." (Hamamatsu area, nurse in a palliative care team)
2. Advantages
[The material promotes communication with patients]
"It is easy for us to communicate with patients who utilize the notebook and write down comments in it frequently. They say, 'I would like to hear any information on my condition from you'. The notebook was also useful when an inpatient was discharged, then readmitted, and discharged recently. I was able to ask the patient about his/her conditions during the period of the first discharge while viewing copies of medical records attached to the notebook." (Hamamatsu area, hospital nurse)
[The material helps patients know that they have learned self-control]
"A large number of patients are always concerned about their blood sampling and other examination data, and want the notebook to include all kinds of health care records. Some patients even ask us: 'Can I have a copy of the test results?' before we provide them with the document. The material helps them check and compare the data to understand their conditions." (Hamamatsu area, hospital nurse)
[The notebook allows health and welfare care professionals to share information]
"One of the advantages of the notebook is that it provides all kinds of information, including questions to a patient, drugs administered, examination results, and other records. It allows us to respond to an emergency case promptly since explanations provided by physicians and nurses for each hospital visit are on the same page. Usually, health insurance-based pharmacies like us cannot obtain patient information other than prescriptions. By reading explanations and instructions that a patient received at discharge or when the treatment course was changed, we can understand the therapeutic strategy developed by the attending physician for the patient." (Hamamatsu area, pharmacist at an insurance-based pharmacy)
"The material allows me to understand the conditions of patients and their situation. It also helps physicians notice what otherwise might be overlooked. Physicians often write down comments, including 'I told a patient not to ride a bicycle', and requests by patients." (Tsuruoka area, home-visiting nurse)
3. Obstacles to its widespread use
[Few advantages for patients/Burden of filling in the notebook]
"Some people do not bother to fill in the notebook. When I visit one of those patients as a home-visiting nurse, I record the patient's condition on that day in the notebook, and show it to his/her family. The patient brings the notebook to the day care center to have a staff member fill it in, and, in hospital, physicians write down their diagnosis in it." (Tsuruoka area, home-visiting nurse)
[There is no point in using the material unless it is used by all health-related organizations in the community]
"As patients receive a variety of documents besides this in hospital, it is difficult for us to explain to them the significance of the material and taking it to a pharmacy and other health professionals. Since some local pharmacies do not know of the material, a patient who brings and shows it to one of them might be asked: 'What is this?' To increase awareness of this notebook among patients, a physician has to provide a patient with a clear explanation while giving a diagnosis: 'I am going to write down the results in this notebook of yours. Show it to a pharmacist', to help the patient convey its purpose to a pharmacist at a local pharmacy." (Hamamatsu area, hospital pharmacist)
[There are already a lot of similar materials]
"Too many notebooks have already been provided. Health care and several types of medication notebooks... I can barely keep up with medication notebooks. These should be integrated into one general notebook for a patient to fill in all kinds of health care information. The ministry should take the initiative to produce and issue such a notebook. In some cases, one patient has two or more medication notebooks." (Kashiwa area, hospital pharmacist)

≪肺癌診療にまつわるトータルサポート——治療とケア≫
緩和ケアの実際

東　陽一郎　　木下寛也*

要旨

- 2012年度から2016年度までの5年間を対象として,「がん対策推進基本計画」の見直しが2012年に行われた. 早期から緩和ケアを推進し, 全人的な緩和ケアを充実させることが目標とされた.
- 最近では, 非切除進行非小細胞肺癌患者において, 早期緩和ケア導入によりQOLの改善や抑うつの低下を認めたとの報告もある.
- 一方, 在宅医療の推進に対して介護保険制度の改正も行われた.
- 「24時間対応の定期巡回・随時対応型の訪問介護看護」と「小規模多機能型居宅介護に, 訪問看護を組み合わせた複合型サービス」が創設され, 診療報酬も改定された.
- がん患者における早期から終末期までの集学的治療に, 切れ目のない緩和ケアの充実が求められている.

がん対策推進基本計画○

「がん対策推進基本計画」は,「がん対策基本法」に基づき2007年6月に1回目の策定が政府により行われた. その後, 2012年度から2016年度までの5年間を対象とし, 2012年6月に見直しが行われた[1]. 重点的に取り組むべき4項目の課題の一つとして,「がんと診断された時から緩和ケアを推進することで, 患者とその家族が精神心理的苦痛に対する心のケアを含めた全人的な緩和ケアを受けられる体制の充実」が掲げられた. また新たに,「働く世代や小児へのがん対策の充実」が追加された. 次に, 緩和ケアに関する分野別施策と個別目標において, ① 今後5年以内にがん診療に携わるすべての医療従事者が基本的な緩和ケアを理解し, 知識と技術を習得すること, ②3年以内に拠点病院を中心に緩和ケアチームや緩和ケア外来の充実を図ることが掲げられた. Table 1に今回の改定内容を記載した.

肺癌患者への早期緩和ケア○

2010年に非切除進行非小細胞肺癌患者への早期緩和ケア導入について, 無作為化比較試験の結果が報告された[2]. 外来患者151例を標準療法群

キーワード：がん対策推進基本計画, 早期緩和ケア, 介護保険制度, 在宅緩和ケア.

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Table 1.「がん対策推進基本計画」の概要

第１．基本方針
・がん患者を含めた国民の視点に立ったがん対策の実施
・重点的に取り組むべき課題を定めた総合的かつ計画的ながん対策の実施
・目標とその達成時期の考え方
第２．重点的に取り組むべき課題
１．放射線療法，化学療法，手術療法の更なる充実とこれらを専門的に行う医療従事者の育成
２．がんと診断された時からの緩和ケアの推進
３．がん登録の推進
４．働く世代や小児へのがん対策の充実
第３．全体目標
１．がんによる死亡者の減少
２．全てのがん患者とその家族の苦痛の軽減と療養生活の質の維持向上
３．がんになっても安心して暮らせる社会の構築
第４．分野別施策と個別目標
１．がん医療
(1) 放射線療法，化学療法，手術療法の更なる充実とチーム医療の推進
(2) がん医療に携わる専門的な医療従事者の育成
(3) がんと診断された時からの緩和ケアの推進
(4) 地域の医療・介護サービス提供体制の構築
(5) 医薬品・医療機器の早期開発・承認等に向けた取組
(6) その他(希少がん，病理診断，リハビリテーション)
２．がんに関する相談支援と情報提供
３．がん登録
４．がんの予防
５．がんの早期発見
６．がん研究
７．小児がん
８．がんの教育・普及啓発
９．がん患者の就労を含めた社会的な問題
第５．がん対策を総合的かつ計画的に推進するために必要な事項(割愛)

今回の見直しで新規に追加された項目を下線で示した．

と早期緩和ケア導入群の２群に無作為化し，治療開始時と 12 週間後に主評価項目として QOL と抑うつの評価を行った．その結果，早期緩和ケア導入群で有意差をもって QOL が改善し，また抑うつの低下が認められた．副次的評価項目ではあるものの，生存期間については標準療法群が8.9ヵ月であるのに対し，早期緩和ケア導入群で11.6ヵ月と有意に延長した．医療環境の異なる日本においても同様の結果がもたらされるかが，今後の課題である．

肺癌患者の症状および治療○

肺癌患者では，根底に肺気腫や間質性肺炎などを合併していることも多い．初発症状には呼吸困難，咳嗽や喘鳴，喀血や胸痛などがあり，非切除進行期でみつかることも多々ある．局所進行性に胸腔内臓器へ浸潤することで上大静脈症候群や嗄声，胸水および心嚢液貯留などから急激に状態が変化しうる．遠隔転移では脳転移，骨転移，肝転移による臓器症状をきたすことが多い．また術後であれば残存肺への再発，そのほかには放射線・抗癌剤などによる治療関連肺障害も経験する．下

記の4項目について，最近の情報に限局して概説する．

1．呼吸器症状

呼吸困難にはステロイド・医療用麻薬（モルヒネ製剤）・抗不安薬が用いられる．また補助的に酸素投与，補液管理・利尿薬，鎮咳薬・去痰薬などが使われる．終末期における在宅酸素療法については，保険適用基準どおりではない症例があるものの，呼吸困難感の改善につながるとされる[3]．そのほか，中枢気道病変に対しては縦隔リンパ節への放射線治療やステント治療がある．また胸水および心嚢液貯留には穿刺除去が行われる．2011年には日本緩和医療学会から，「がん患者の呼吸器症状の緩和に関するガイドライン」が発表された[4]．

2．がん疼痛

2010年に日本緩和医療学会から，「がん疼痛の薬物療法に関するガイドライン」が発表された[5]．また，2007年に同学会から厚生労働省に単味のoxycodone hydrochloride hydrate注射剤の開発要望が出されていたが，2012年から使用可能となった．さらに2010年にtramadol hydrochlorideの単味内服薬が使用可能となった．2011年にacetaminophenとの配合錠が出たが，同剤は非がん疼痛にのみ保険適用とされている．

3．中枢神経症状

脳浮腫についてはステロイドやグリセリン製剤が用いられる．そのほか，放射線治療としてγ-ナイフや全脳照射などがある．転移性脳腫瘍に由来する痙攣に対しての治療法は確立されていないが，2010年に日本神経学会から出された「てんかん治療ガイドライン2010」[6]などを参考に薬物療法が行われる．

4．骨転移に伴う症状

zoledronic acidは破骨細胞による骨吸収を抑制するビスホスホネート製剤であるが，肺癌433例を含む転移性骨腫瘍773例において，プラセボ群との無作為化二重盲検試験が行われた．zoledronic acid投与群で骨関連事象の発生頻度が低く，発生までの期間が有意に延長した[7]．また，zoledronic acidに対する完全ヒト型抗RANKL中和抗体の非劣性が報告され[8]，2012年より使用可能となった．緩和的放射線治療については骨転移のみならず脳転移・上大静脈症候群などでも対象となり[9]，薬物による癌疼痛管理と併用して行われる．

介護保険制度改正○

在宅医療の推進について，介護保険制度の改正が2012年に行われた．改正のポイントとしては，①中重度者や医療ニーズの高い要介護者への対応強化，②コスト高の施設から居住系サービスへの移行促進，③独居世帯や老老世帯を支える新サービス創設，④認知症ケア促進，⑤高齢者が安心して暮らせる住まいの整備を目標としたことがあげられる[10]．

在宅緩和ケア○

がん終末期における病院死と在宅死を比較した研究については，学会誌ではないものの症例対照研究が報告されている[11]．大学病院死32例と在宅死14例における終末期の比較が行われ，在宅死群では意識障害を認める期間が有意に短く，また最終経口摂取の時期は死亡する時期に有意に近いことが報告された．次に，肺癌患者への在宅緩和ケアに関しては，在宅緩和ケアとして訪問診療を行った肺癌群152例および非肺癌群401例について，在宅緩和ケアががん終末期に療養形態の選択肢となりうるかを検討した論文が報告されている[12]．2003年から5年間，単一施設における2群間の後方視的解析であり，①在宅緩和ケアの対象として肺癌群と非肺癌群に相違があるか，②在宅死は可能か，③在宅緩和ケアは質の高い医療・ケアであるかが検討された．①については，男性が両群ともやや多く，肺癌群に呼吸困難の発症頻度が高かった．また肺癌群の訪問期間については，30日以内が48.8%，31～90日が33.3%を占めており，両群間で大きな差を認めなかった．

②については，在宅死した肺癌群129例と非肺癌群344例について，提供するケアの問題などで入院せざるをえなかった症例をもとに在宅死率を，また抗癌剤治療を諦められないなどの理由で中断した症例をもとに在宅完結率が算出された．その結果，肺癌群の在宅死率が96.3%，在宅完結率が89.0%となり，それぞれ非肺癌群に差はないとされた．③については，訪問診療開始時と死亡前1週間のADL，コミュニケーションの変化，医療用麻薬の使用量の変化から苦痛症状の緩和が検討された．死亡2日前と前日でトイレ移動ができていた割合や言語による意思疎通ができていた割合も，おおむね同程度であった．医療用麻薬の使用量も肺癌群85例と非肺癌群223例で検討され，両群で疼痛・呼吸困難などへの症状緩和の困難さは認めないとされた．また，2009年には日本緩和医療学会から，「在宅緩和ケアガイドブック」が出版されている．

おわりに

「がん対策推進基本計画」の見直しや介護保険制度の改正が，2012年に行われた．各種症状に対するガイドラインが学会から出され，新規薬剤も登場している．肺癌にかかわらず，がん患者における早期から終末期までの集学的治療に，切れ目のない緩和ケアの充実が求められている．

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"S'ils n'ont pas de pain, qu'ils mangent de la brioche." Focus on "Anaerobic respiration sustains mitochondrial membrane potential in a prolyl hydroxylase pathway-activated cancer cell line in a hypoxic microenvironment"

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IN THEIR ARTICLE, published in this issue of the *American Journal of Physiology-Cell Physiology*, Takahashi and Sato (7) point to the possibility that anaerobic respiration depends on complex II activity in mammalian cells, especially cancer cells. Fumarate respiration is established in parasites and shellfish but has not been unequivocally demonstrated in mammalian cells. What cancer cells are eating to generate energy for survival without oxygen and glucose is one challenging subject.

Cancer cells depend on glycolysis for their energy production, even during sufficient oxygen supply, and this dependence on glycolysis for energy production, i.e., the Warburg effect, has long been believed to be one of the most general characteristics of cancer (8). On the other hand, tumor angiogenesis is also strongly activated in most cancer tissues through activation of hypoxia-inducible factor (HIF)-1-dependent and -independent pathways, and tumor angiogenesis is often closely associated with poor prognosis for patients with various types of cancer (5). However, many clinical investigations have revealed that most cancer tissues are strongly hypoxic, despite vigorous angiogenesis and the preference of cancer cells for glycolysis. Therefore, cancer tissue hypoxia is assumed to be a result of insufficient blood supply. Tumor vasculature might be subjected to structural distortion by continuous tumor growth and death, resulting in structural and functional immaturity. The main energy production pathway of mammalian cells in an anaerobic environment is believed to be glycolysis, but, in the case of tumor hypoxia, glucose and other nutrient supplies might also be limited. Information about how cancer cells produce energy to maintain their proliferation and/or life under such harsh conditions is limited (2, 3).

Several authors have proposed the possibility of anaerobic respiration by renal cells and cancer cells, but its biochemical mechanisms and significance remain to be established (5, 6). Takahashi and Sato (7) used an elegant experimental system to demonstrate the possibility of an alternative anaerobic respiration. They developed a two-dimensional tissue model in which a monolayer of cultured cells expressing green fluorescent protein was placed under a coverslip so that oxygen is supplied only from the edge of the coverslip. In this system, an oxygen gradient was formed and visualized as the red shift of fluorescence, which depends on oxygen tension (6). When mitochondrial membrane potential was also visualized by a cationic fluorescent dye, the point at which the oxygen supply became limiting for mitochondrial function could be established. Using this system, they showed that the maximum distance for diffusion of oxygen was ~500 μm from the oxygen source (the edge of the coverslip), at which point mitochondrial membrane potential was abolished [the anoxic front (AF)]. When prolyl hydroxylase domain-containing proteins were inhibited by dimethyloxalylglycine (DMOG), the AF was extended to 1,500–2,000 μm, and the effect was much more prominent in a cancer cell line than in a fibroblast-like cell line. DMOG pretreatment significantly reduced tissue oxygen gradients, indicating sustained mitochondrial membrane potential with reduced respiration. In addition, DMOG effects were completely abolished by pharmacological inhibition of complex II, but not complex III, suggesting that complex II (probably with complex I) sustains mitochondrial membrane potential in the absence of oxygen (anaerobic respiration) in cells in which PHD activity is inhibited.

Recently, the importance of α-ketoglutarate-dependent dioxygenases in biology has been widely accepted, and this is especially true for the HIF-1 pathway in cancer and epigenetic regulation of cellular function. In the work of Takahashi and Sato (7), DMOG was used to activate hypoxic adaptation, and "cellular anaerobic respiration" was found to be activated. HIF-1 is the most important and well-studied transcription factor regulating a wide variety of cellular adaptations to the hypoxic environment. However, α-ketoglutarate-dependent dioxygenases include a large number of family members, and DMOG is not highly specific for the prolyl hydroxylases that regulate HIF-1α. The recent discovery of isocitrate dehydrogenase mutations, which produce large increases in the levels of the "onco-metabolite" D-2-hydroxyglutarate, raised the following question: How does this metabolite exert its oncogenic effect? HIF-1 activation is a strong candidate, but more extensive genetic and biochemical analyses, including effects on TET dioxygenases, histone demethylases, and prolyl hydroxylases, are needed to explore the biological relevance of D-2-hydroxyglutarate (1) and the work of Takahashi and Sato (7).

The work of Takahashi and Sato (7) points to the possibility that anaerobic respiration depends on complex II activity in mammalian cells, especially cancer cells, and this is one of the most interesting possibilities. Fumarate respiration is established in parasites and shellfish but has not been unequivocally demonstrated in mammalian cells (4). What cancer cells are eating to generate energy for survival without oxygen and glucose is one challenging subject. Additional genetic and biochemical studies are needed.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

H.E. drafted the manuscript; H.E. approved the final version of the manuscript.

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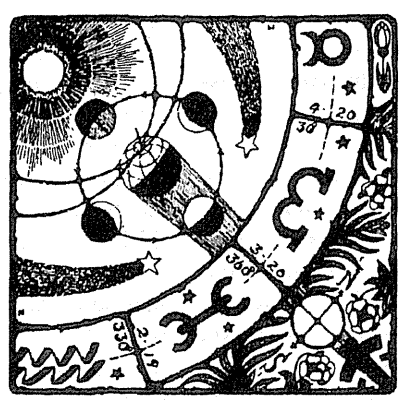

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ORIGINAL ARTICLE

Metabolomic profiling of lung and prostate tumor tissues by capillary electrophoresis time-of-flight mass spectrometry

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Abstract Metabolic microenvironment of tumor cells is influenced by oncogenic signaling and tissue-specific metabolic demands, blood supply, and enzyme expression. To elucidate tumor-specific metabolism, we compared the metabolomics of normal and tumor tissues surgically resected pairwise from nine lung and seven prostate cancer patients, using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS). Phosphorylation levels of enzymes involved in central carbon metabolism were also quantified. Metabolomic profiles of lung and prostate tissues comprised 114 and 86 metabolites, respectively, and the profiles not only well distinguished tumor from normal tissues, but also squamous cell carcinoma from the other tumor types in lung cancer and poorly differentiated tumors from moderately differentiated tumors in prostate cancer. Concentrations of most amino acids, especially branched-chain amino acids, were significantly higher in tumor tissues, independent of organ type, but of essential amino acids were particularly higher in poorly differentiated than moderately differentiated prostate cancers. Organ-dependent differences were prominent at the levels of glycolytic and tricarboxylic acid cycle intermediates and associated energy status. Significantly high lactate concentrations and elevated activating phosphorylation levels of phosphofructokinase and pyruvate kinase in lung tumors confirmed hyperactive glycolysis. We highlighted the potential of CE-TOFMS-based metabolomics combined with phosphorylated enzyme analysis for understanding tissue-specific tumor microenvironments, which may lead to the development of more effective and specific anticancer therapeutics.

Keywords Metabolomics · CE-MS · Phosphoproteomics · Lung cancer · Prostate cancer · Tumor microenvironment

Kenjiro Kami and Tamaki Fujimori contributed equally to this study.

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1 Introduction

Hyperactivity of glycolysis independent of oxygen availability is a hallmark of cancer metabolism (Warburg effect) (Warburg 1956). Glycolytic energy metabolism of tumor cells is advantageous for perpetual proliferation and meeting the high demand for non-essential amino acids, fatty acids, and nucleotides, although not for efficient production of ATP. Besides glucose, glutamine is significantly consumed by most tumor cells and metabolized to

alanine, lactate, and ammonium ions, which are secreted out of the cells, in a process called glutaminolysis (Heiden et al. 2009). Corroborating these features of cancer metabolism, our previous metabolome analyses of colon and stomach tumor tissues, using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS), have revealed significantly high tumor concentrations of glycolytic intermediates including lactate, tricarboxylic acid (TCA) cycle intermediates, and amino acids (Hirayama et al. 2009). Moreover, inter-organ metabolomic differences were more significant than normal-versus-tumor differences within the same organ, which revealed the complexity in generalizing a tumor-specific, organ-independent metabolic profile. This suggested that cells alter their metabolism along with tumorigenesis while retaining much of the metabolism that is unique to their organs of origin. To test this hypothesis further and gain an insight into cancer metabolism, we analyzed metabolomic profiles of normal and tumor tissues obtained from lung and prostate cancer patients.

Deciphering the difference in the flow of energy metabolism between cancer and normal cells solely from the tissue metabolome data is often difficult. The activities of most glycolytic enzymes are known to be regulated by phosphorylation; therefore, we used nano-liquid chromatography-tandem mass spectrometry (nanoLC–MS/MS) to quantify phosphorylation levels of 13 sites contained in ten selected enzymes involved in glycolysis and the TCA cycle. The results indicate that tumor metabolomic profile is highly dependent on its organ of origin, and exhibits unique patterns dependent on cancer type as well as differentiation status. This demonstrates the potential of CE-TOFMS-based metabolomics complemented by phosphorylated enzyme analysis for gaining further insight into the complexity and heterogeneity of tumor metabolism.

2 Materials and methods

2.1 Sampling and metabolite extraction

All the experiments were conducted according to the study protocol that was approved by the Institution Review Board of the National Cancer Center, Japan. Informed consent was obtained from all the participants.

Tumor and surrounding tissues were surgically resected from nine lung and seven prostate cancer patients, who had been administered with no anticancer drugs or medications that could greatly modify their metabolisms previous to the surgical treatments. Clinical information on the patients is listed in Table 1. The resected tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C until metabolite extraction. Sample tissues were weighed and completely homogenized by multi-beads shocker (Yasuikikai, Osaka, Japan) at 2,000 rpm for 3 min, after adding 0.5 ml ice-cold methanol containing 50 μM methionine sulfone and camphor-10-sulfonic acid as internal standards. The homogenates were mixed with 0.5 ml chloroform and 0.2 ml ice-cold Milli-Q water. After centrifugation at 2,300×g for 5 min, the supernatant was centrifugally filtrated through 5-kDa cut-off filters (Millipore, Bedford, MA, USA) at 9,100×g for 3 h to remove proteins. The filtrate was centrifugally concentrated in a vacuum evaporator, dissolved with Milli-Q water, and analyzed by CE-TOFMS.

Table 1 Clinicopathological information of patients and their tumor tissues. W, M, and P in the differentiation status indicate well-, moderately-, and poorly- differentiated tumors, respectively

Organ	ID	Age	Sex	Type	Stage	Differentiation
Lung	L1	82	Male	Squamous cell carcinoma	2B	M
	L2	82	Male	Squamous cell carcinoma	1B	M
	L3	77	Male	Squamous cell carcinoma	1B	P
	L4	80	Female	Adenocarcinoma	1B	M
	L5	78	Male	Pleomorphic carcinoma	3B	N/A
	L6	81	Male	Adenocarcinoma	1A	W
	L7	56	Male	Squamous cell carcinoma	3B	M–P
	L8	61	Male	Large cell carcinoma	1B	N/A
	L9	57	Male	Adenocarcinoma	1B	P
Prostrate	P1	68	Male	Adenocarcinoma	2	M
	P2	66	Male	Adenocarcinoma	2	P
	P3	67	Male	Adenocarcinoma	2	P
	P4	63	Male	Adenocarcinoma	3	P
	P5	62	Male	Adenocarcinoma	2	M
	P6	65	Male	Adenocarcinoma	2	M
	P7	58	Male	Adenocarcinoma	2	M

2.2 CE-TOFMS analysis and data processing

CE-TOFMS analysis was performed by an Agilent CE system combined with a TOFMS (Agilent Technologies, Palo Alto, CA, USA) as described previously (Ohashi et al. 2008) with slight modifications. Cationic metabolites were separated through a fused silica capillary (50 μm internal diameter × 80 cm total length) preconditioned with a commercial buffer (H3301-1001, Human Metabolome Technologies Inc. (HMT), Tsuruoka, Japan) and filled with 1 M formic acid as electrolyte, and a commercial sheath liquid (H3301-1020, HMT) was delivered at a rate of 10 μl/min. Sample solution was injected at a pressure of 50 mbar for 10 s. The applied voltage was set at 30 kV. Electrospray ionization-mass spectrometry (ESI-MS) was conducted in the positive-ion mode and the capillary and fragmentor voltages were set at 4,000 and 120 V, respectively. Nebulizer pressure was configured at 5 psig and N_2 was delivered as a drying gas at a rate of 7 l/min at 300 °C. Exact mass data were acquired at the rate of 1.5 cycles/s over a 50–1,000 *m/z* range. Anionic metabolites were analyzed also through the fused silica capillary preconditioned with a commercial buffer (H3302-1022, HMT) and filled with 50 mM ammonium acetate solution (pH 8.5) as electrolyte, and the aforementioned sheath liquid was delivered at a rate of 10 μl/min. Sample solution was injected at a pressure of 50 mbar for 6 s. The nebulizer pressure, drying gas and its flow rate, applied voltage, and scanning condition of the spectrometer were configured in the same manner as the cationic metabolite analysis. ESI-MS was conducted in the negative mode, and the capillary and fragmentor voltages were set at 3,500 and 125 V, respectively. The data obtained by CE-TOFMS analysis were preprocessed using our proprietary automatic integration software, MasterHands. Each metabolite was identified and quantified based on the peak information including *m/z*, migration time, and peak area. The quantified data were then evaluated for statistical significance by Wilcoxon signed-rank test.

2.3 Enrichment of phosphopeptides

Sample tissues were disrupted by multi-beads shocker and suspended in 100 mM Tris–HCl (pH 9.0) containing 8 M urea, protein phosphatase inhibitors and protein phosphatase inhibitors cocktails (Sigma, St. Louis, MO, USA). After centrifugation at 1,500×*g* for 10 min, the supernatant was reduced with 1 mM dithiothreitol, alkylated with 5 mM iodoacetamide, and then digested with Lys-C endopeptidase at 37 °C for 3 h, followed by 5-fold dilution with 50 mM ammonium bicarbonate and digestion with trypsin at 37 °C overnight. The digested samples were desalted using StageTips with SDB-XC Empore disk membranes (3 M, St. Paul, MN, USA) (Rappsilber et al. 2003).

Phosphopeptides were enriched with hydroxy acid-modified metal oxide chromatography (HAMMOC) (Kyono et al. 2008; Sugiyama et al. 2007). Briefly, custom-made metal oxide chromatography tips were prepared using C8-StageTips and titania beads as described previously (Rappsilber et al. 2007). Prior to loading samples, the tips were equilibrated with 0.1 % trifluoroacetic acid (TFA), 80 % acetonitrile and 300 mg/ml lactic acid (solution A). The digested samples from normal or tumor tissues were diluted with 100 μl solution A and loaded into the HAMMOC tips. After successive washing with solution A and solution B (0.1 % TFA and 80 % acetonitrile), 0.5 % piperidine was used for elution. The eluted fraction was acidified with TFA, desalted using SDB-XC-StageTips, and concentrated in a vacuum evaporator, followed by the addition of solution A for subsequent nanoLC–MS/MS analysis. The phosphopeptide enrichment and sample pretreatment was conducted in duplicate.

2.4 NanoLC–MS/MS analysis and database search

NanoLC–MS/MS analyses were conducted using LTQ-Orbitrap (Thermo Fisher Scientific, Rockwell, IL, USA), a Dionex Ultimate 3000 pump (Thermo Fisher Scientific) and an HTC-PAL autosampler (CTC Analytics, Zwingen, Switzerland). A self-pulled needle (150 mm length × 100 μm internal diameter, 6-μm opening) packed with ReproSil C18 materials (3 μm, Dr. Maisch, Ammerbuch, Germany) was used as an analytical column with "stone-arch" frit (Ishihama et al. 2002). A polytetrafluoroethylene-coated column holder (Nikkyo Technos, Tokyo, Japan) was mounted on an *x–y–z* nanospray interface, and a tee connector with a magnet was used to hold the column needle and to set the appropriate spray position. The injection volume was 5 μl and the flow rate was 500 nl/min for the gradient separation of peptides (Ishihama 2005). The mobile phases consisted of (A) 0.5 % acetic acid and (B) 0.5 % acetic acid and 80 % acetonitrile. A three-step linear gradient of 5–10 % B in 5 min, 10–40 % B in 60 min, 40–100 % B in 5 min and 100 % B in 10 min was used. A spray voltage of 2,400 V was applied via the tee connector. The MS scan range was *m/z* 300–1,500 and the top ten precursor ions were selected for subsequent MS/MS scans. Resolution setting and its maximum injection time were configured at 60,000 and 500 ms, respectively. We also configured the normalized collision energy at 35.0, the isolation width at two, and the minimum signal at 500. Automatic gain controls were set at 500,000 in the MS analysis and at 10,000 in the MS/MS analysis. The capillary temperature was set at 200 °C. A lock mass function was used with a peak derived from polydimethylsiloxane

as a lock mass for the LTQ-Orbitrap to obtain constant mass accuracy during gradient analysis (Olsen et al. 2005). Mass Navigator version 1.2 (Mitsui Knowledge Industry, Tokyo, Japan) was used to create peak lists on the basis of the recorded fragmentation spectra. Peptides and proteins were identified by means of automated database searching using Mascot (Matrix Science, London, UK) against UniProt/Swiss-Prot.

3 Results and discussion

3.1 Overall metabolomic profile and amino acids

We analyzed metabolomic profiles of normal and tumor tissues obtained from nine lung and seven prostate cancer patients by using CE-TOFMS. Based on their *m/z* values and migration times, 114 and 86 metabolites were measured in the lung and prostate tissues, respectively (Supplementary Table S1), and visualized on a metabolome-wide pathway map (Supplementary Fig. S1) using VANTED software (Junker et al. 2006). The metabolomic data were then normalized and hierarchically clustered on both the metabolite and sample axes for a heat map representation (Supplementary Fig. S2) and further analyzed by principal component analysis (PCA) using MeV software (Saeed et al. 2003). Thirty-nine metabolites including glycolytic and TCA cycle intermediates, amino acids, and purine nucleoside phosphates, were absolutely quantified (Supplementary Table S2). PCA indicated that tumor metabolomic profiles were much more heterogeneous than their normal counterparts and comprised multiple clusters (Fig. 1a). With reference to the patient information (Table 1) and the hierarchically-clustered samples (Supplementary Fig. S2), tumor types appeared to play a greater part than tumor stage or differentiation status in altering the overall metabolomic profile in lung cancer, whereas differentiation status contributed more in prostate cancer. Indeed, the cluster of squamous cell carcinoma (SCC) patients (L1–3 and L7) was well-distinguished from that of adenocarcinoma (L4, L6 and L9) and pleomorphic carcinoma (L5). This may reflect the intrinsic pathological difference that adenocarcinoma cells but not squamous carcinoma cells retain their function of secreting mucus as glandular epithelial cells. In prostate samples, poorly differentiated prostate tumors (P2–4) were distant from the cluster of moderately differentiated (P1 and P5–7) tumors, which overlapped with that of normal samples. This may be due to higher duct-forming capacity and hormone response of well-differentiated prostate tumors, as well as normal prostate cells, than that of poorly differentiated tumors.

Both lung and prostate tumor samples were well-separated primarily along the PC1 axis; thus, factor loadings for the PC1 axis were evaluated. Correlations with the PC1 were particularly high in branched-chain amino acids (BCAAs) such as Val ($R = -0.97$), Ile (-0.97), and Leu (-0.89) in lung, and Leu (-0.87) in prostate samples (Supplementary Fig. S3). BCAAs are known to be avidly taken up by tumors and highly oxidized in cancer patients (Baracos and Mackenzie 2006), and thus may serve as effective indicators for diagnosing lung tumors. In fact, average lung tumor concentrations of all the 19 amino acids measured were higher than their respective normal levels, as were average prostate tumor levels of all the amino acids except Asp, Ile and Met (Fig. 1b). This is possibly due to hyperactivity of protein degradation and amino acid transporters in tumor cells (Fuchs and Bode 2005; Vander Heiden et al. 2009). Although average tumor levels of most amino acids in lung samples were significantly higher than their respective normal levels, normal and tumor Asp levels were comparable. Asp may be actively consumed as a precursor for nucleic acids and these TCA cycle intermediates, because tumor concentrations of malate, fumarate and succinate were significantly higher than the normal levels. In prostate tissues, levels of some amino acids such as Asn, Lys, Phe, Ser and Tyr and total essential amino acids were particularly higher in poorly differentiated tumors (P2–4; black in Fig. 1b) than moderately differentiated tumors (P1 and P5–7; gray in Fig. 1b), of which levels were comparable to the corresponding normal levels (Fig. 1b), implying enhancement of acquiring the amino acids upon dedifferentiation of prostate cancer cells.

3.2 Energy charge and adenosine and guanosine phosphates

Adenylate and guanylate energy charges (([RTP] + 0.5 × [RDP])/([RTP] + [RDP] + [RMP]), R = A or G) were lower in lung tumor than normal tissues (Fig. 2a); however, tumor levels were significantly higher than normal levels for ATP (3.8-fold), GTP (4.2-fold), and the other adenosine and guanosine phosphates (1.9–7.9-fold), and hence total adenylates (4.5-fold) and guanylates (4.0-fold). Tumor concentrations of these metabolites were relatively higher in all the SCC samples (L1–3 and L7; black in Fig. 2a) than the others (gray in Fig. 2a) such as L5 and L6, whose overall tumor metabolomic profiles resembled their respective normal profiles (Fig. 1). Purine synthesis may thus be hyperactivated in lung tumors, especially SCC, probably with a high basal ATP ↔ ADP turnover and purine salvage for maximizing their growth. Although prostate tissues showed much less normal-versus-tumor

differences, tumor ADP level was significantly lower than normal level (Fig. 2b). High absolute concentrations of ADP and GDP among other purine nucleoside phosphates are unique to prostate tissues, and ATP and AMP levels were relatively higher in poorly differentiated tumors (P2–4; black in Fig. 2b) than moderately differentiated tumors (P1 and P5–7; gray in Fig. 2b). This might be due to a differential expression of adenylate kinase catalyzing the reaction, 2ADP ↔ ATP + AMP, which is undetectable in adult prostate but shows activity along with its malignant alteration (Hall et al. 1985).

3.3 Glycolytic and TCA cycle intermediates and phosphorylated enzymes

Most glycolytic and TCA cycle intermediates were absolutely quantified (Fig. 3a), and phosphorylation levels of associated enzymes were also examined (Fig. 3b). Tumor lactate levels were higher than normal levels in both lung and prostate tissues, indicating their enhanced glycolysis and lactate fermentation, which reaffirmed the Warburg effect in cancer. Lung tumor levels of fructose 6-phosphate and fructose 1,6-bisphosphate were significantly lower and higher, respectively, than their corresponding normal levels. This may be partly explained by significantly high tumor levels of S386 phosphorylation in phosphofructokinase, which enhances its activity (Brand and Soling 1975), and thus the overall glycolytic flux because it is a bottleneck enzyme. Although tumor levels of S83 phosphorylation in glyceraldehyde 3-phosphate dehydrogenase and S203 in phosphoglycerate kinase-1 were significantly higher than their respective normal levels, their functional impacts are unknown. Tumor level of S37 phosphorylation of pyruvate kinase, which enhances its activity (Le Mellay et al. 2002), was significantly higher than the normal level. This may rationalize the trend that phosphoenolpyruvate and pyruvate were significantly lower and higher, respectively, in tumor than normal tissues. Tumor levels of S293 and S291 phosphorylation in pyruvate dehydrogenase, which inhibit its activity (Korotchkina and Patel 2001; Patel and Korotchkina 2001), were significantly higher than normal levels in all the lung cancer patients, except L6. This inhibition may contribute to the enhanced glycolysis and resulting lactate accumulation in lung tumors. In prostate tissues, however, most glycolytic intermediates were not detected, probably owing to inevitable overdilution of the samples for reducing polyamine concentrations, which otherwise adversely interfere with CE-TOFMS analysis. Trivial differences were observed between normal and tumor prostate phosphorylation levels of most glycolytic enzymes except glucose 6-phosphate isomerase (G6PI); nevertheless, the impact of elevated phosphorylation on the activity of G6PI is uncertain. Although intriguing, there was no apparent correlation between significantly high tumor levels of S481 phosphorylation in ATP citrate lyase in SCC samples, L1, L3

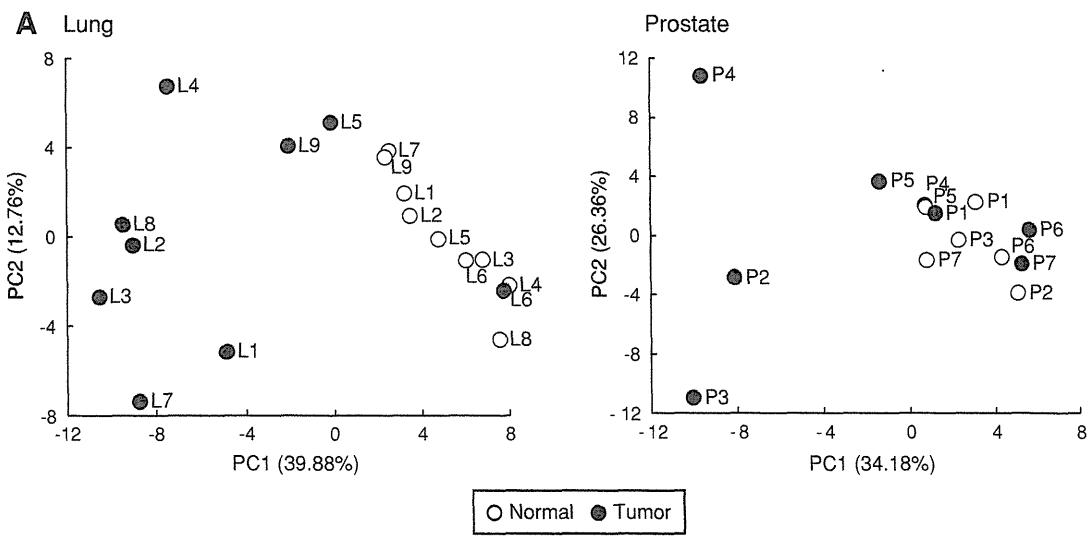
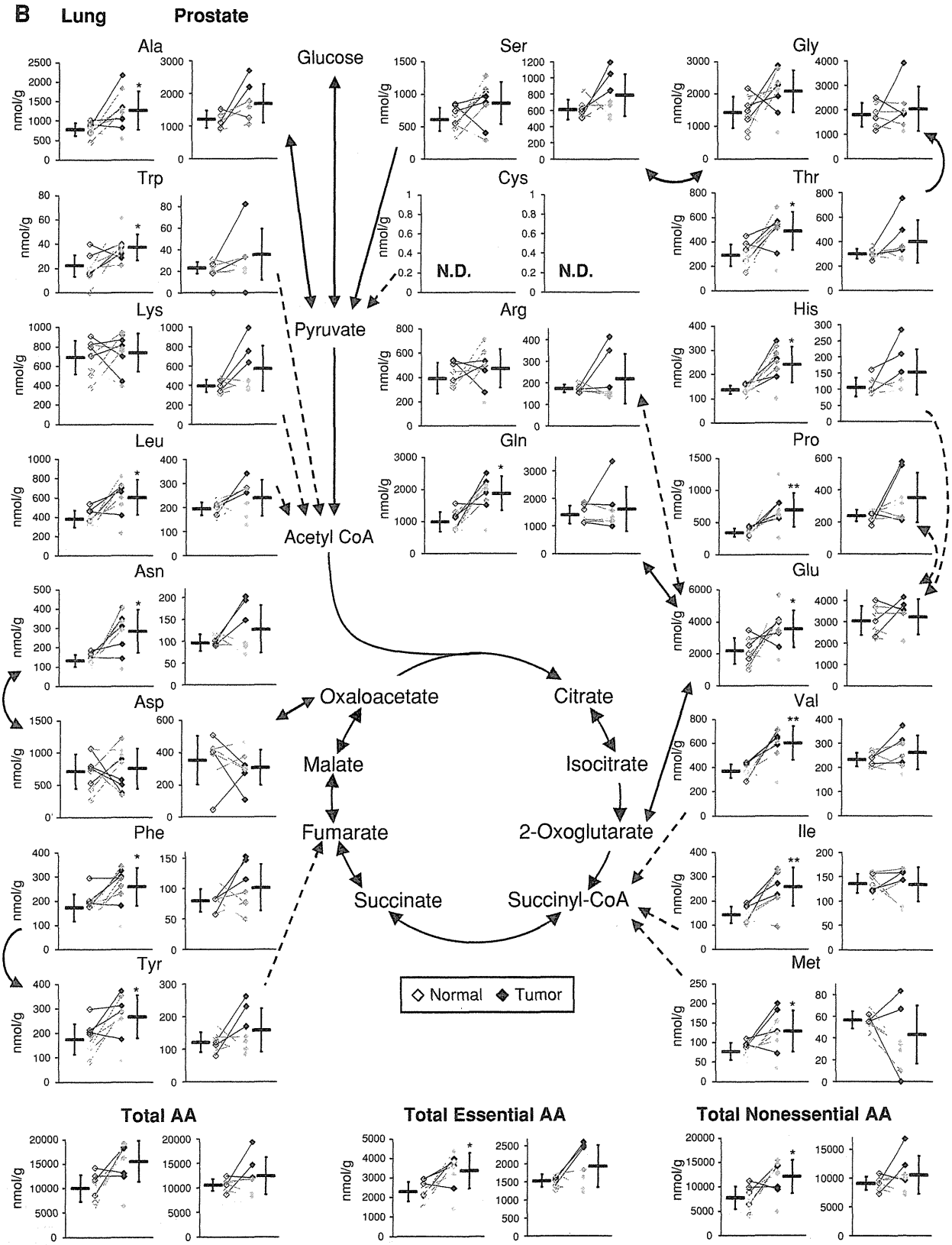


Fig. 1 **a** Score plots of PCA using the normalized metabolomic data of paired normal and tumor tissues obtained from lung (*left*) and prostate (*right*) cancer patients. The sample codes correspond to the patient IDs listed in Table 1. *Percentage values* indicated on the axes represent the contribution rate of the first (PC1) and the second (PC2) principal components. **b** Quantified levels of amino acids in normal (*left, open dots*) and tumor (*right, filled dots*) tissues obtained from lung and prostate cancer patients. *Horizontal bars* represent mean ± SD of normal (*left*) and tumor (*right*) samples and each connected pair represents the values for the same patient. *Gray dots* represent the values for patients with non-SCC lung cancer (L4–L6, L8 and L9) and patients with moderately differentiated prostate cancer (P1 and P5–7). *N.D.* indicates that the metabolite level was below the detection limit of the analysis. *Asterisks* indicate the significant differences between normal and tumor tissue levels based on the Wilcoxon signed-rank test (*$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$)



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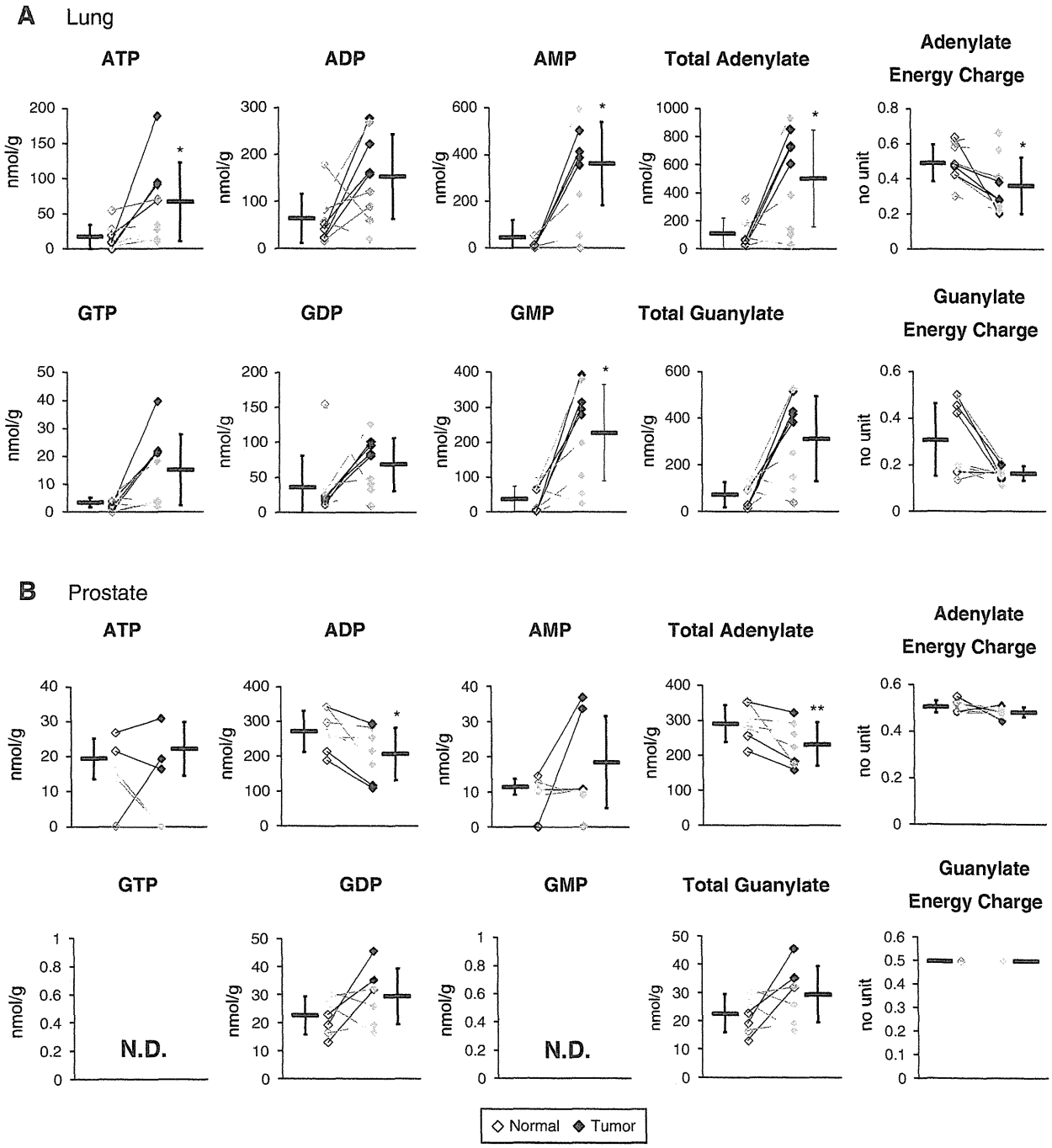
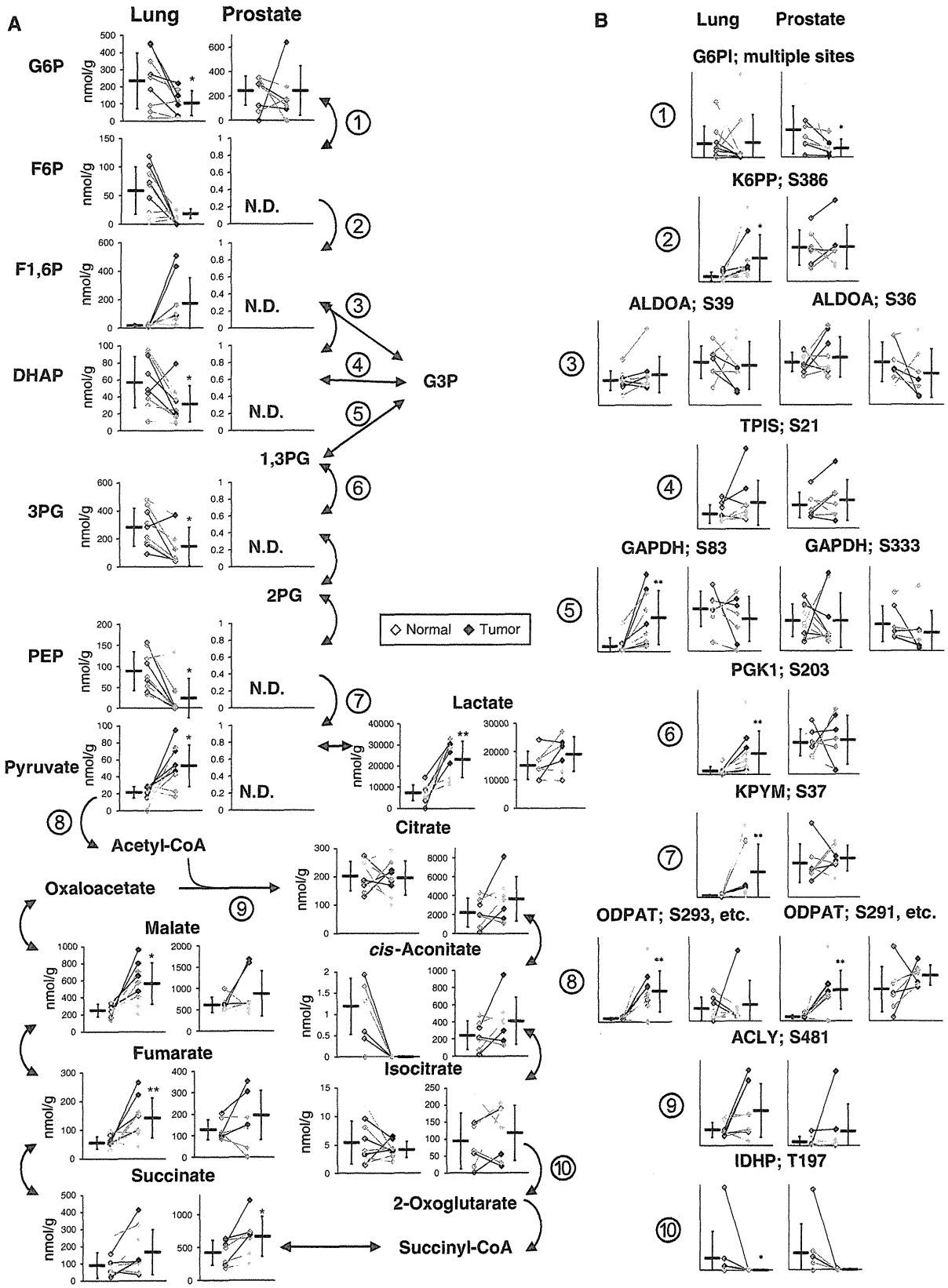


Fig. 2 Adenosine and guanosine phosphates, total adenylates and guanylates, and adenylate and guanylate energy charges of normal (*left, open dots*) and tumor (*right, filled dots*) tissues obtained from lung (**a**) and prostate (**b**) cancer patients. *Horizontal bars* represent mean ± SD of normal (*left*) and tumor (*right*) samples and each connected pair represents the values for the same patient. *Gray dots* represent the values for patients with non-SCC lung cancer (L4–L6, L8 and L9) and patients with moderately differentiated prostate cancer (P1 and P5–7). *N.D.* indicates that the metabolite level was below the detection limit of the analysis. *Asterisks* indicate the significant differences between normal and tumor tissue levels based on the Wilcoxon signed-rank test ($*p < 0.05$; $**p < 0.01$; and $***p < 0.001$)


A
Lung
Prostate
G6P
F6P
F1,6P
DHAP
G3P
1,3PG
3PG
2PG
PEP
Pyruvate
Lactate
Citrate
Acetyl-CoA
Oxaloacetate
Malate
cis-Aconitate
Fumarate
Isocitrate
Succinate
2-Oxoglutarate
Succinyl-CoA
N.D.
nmol/g
◇ Normal ◆ Tumor
B
Lung
Prostate
G6PI; multiple sites
K6PP; S386
ALDOA; S39
ALDOA; S36
TPIS; S21
GAPDH; S83
GAPDH; S333
PGK1; S203
KPYM; S37
ODPAT; S293, etc.
ODPAT; S291, etc.
ACLY; S481
IDHP; T197