られるが、低線量領域ではAEC機能を有効に活用出来ていない場合もあるので、付加フィルターなどを用いるなどの工夫が必要である[7]。

技師による一次チェックは15％であり、内容については、電子カルテ上に記載する施設もあり、施設によってかなり差がみられた。このような現状を把握しながら、今後の認定技師講習会も進めていく必要がある。

まとめ

今回の実態調査結果で、CT検診の低線量の現状、一次チェックの現状などを明確にすることができた。このような実態調査は重要であり、継続して調査を続ける必要がある。さらに、認定取得後の現状についても調査を進めることは、認定技師講習会が低線量化に貢献しているか確認する上でも有用と考える。

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文　献

[1] NPO法人　肺がんCT検診認定技師ホームページ．Update 2010/09/22. http://www.ct-kensin-nintei.jp/info/index.html

[2] 花井耕造、柿沼龍太郎、江口研二、他．肺がんCT検診認定技師の要件－撮影およびスクリーニングにおけるminimum requirement－．日本CT検診学会誌．2007; 14: 122-129

[3] 花井耕造、五味志穂、清水恵子、他．肺がんCT検診認定制度について．日本放射線技術学会誌．2008; 64: 721-724.

[4] 花井耕造、佐藤昌憲、跡田直利、他．肺がんCT検診認定制度の動向－検診に携わる放射線技師の認定について－．日本CT検診学会誌．2009; 16: 150-153

[5] 五味志穂、花井耕造、村松禎久、他．胸部CTスクリーナーアンケート調査結果報告．日本CT検診学会誌．2006; 131: 181-184

[6] 科学的根拠に基づくがん検診推進のページ　がん検診ガイドライン肺がん．Update 2010/9/23. http://canscreen.ncc.go.jp/guideline/haigan.html

[7] Shiho Gomi, Yoshihisa Muramatsu, Shinsuke Tsukagoshi, et al. Low-dose CT screening for lung cancer with automatic exposure control: phantom study. Radiological Physics and Tecnology. 2008; 1: 224-250

大学における定期健康診断時の胸部X線検査のあり方を考える

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飛田　涉，髙梨　信吾，寺尾　英夫
小倉　俊郎，武蔵　学，長尾　啓一

東北大学保健管理センター
弘前大学保健管理センター
大分大学保健管理センター
岡山大学保健管理センター
北海道大学保健管理センター
千葉大学総合安全衛生管理機構

報　告

大学における定期健康診断時の胸部X線検査のあり方を考える

飛田　渉[1]　髙梨　信吾[2]　寺尾　英夫[3]
小倉　俊郎[4]　武蔵　学[5]　長尾　啓一[6]

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はじめに

平成13年9月に開催された第13回国立大学等保健管理施設協議会（平成16年4月より国立大学法人保健管理施設協議会）総会において，健康診断時の胸部X線撮影時における被曝線量について検討する目的で「胸部X線撮影におけるX線被曝線量調査研究班」（班長野原隆彦）が立ち上げられた。これまでの調査研究によって，年1回の健康診断時のこれらの被曝線量で健康障害を起こすことは考えられないものの，被曝量の低減の努力を継続的に行う必要があることが示された[1,2]。

一方，大学における健康診断は学校保健安全法[3]の第13条に基づき全学年に対して毎年実施する必要がある。しかし，検査項目の中で結核の有無については平成17年4月の同施行規則の改訂[4]により，それまでの「大学の全学年」より「大学の第一学年」に変更された。したがって胸部X線検査も「大学の第一学年」のみで良いということになる。しかしながら，木村らの全国の国立大学法人校を対象とした調査結果によると[5]，新規の活動性肺結核患者の発見数は新入生よりも新入生以外の学年において多く，留学生においてはさらに高頻度に新規の活動性肺結核患者の発見例があることより，全国一律に「大学の第一学年」のみにおいて胸部X線検査を行うものとするという改訂に問題があるとしている。八田らの学生数4,000人以上の大学を対象とした調査結果でも同様の結果が得られている[6]。国立大学法人保健管理施設協議会によるこれまでの学生の健康白書をみても定期健康診断時に1年生以外に活動性肺結核が発見されている[7−12]。従って大学生における定期健康診断時の胸部X線検査は「大学の第一学年」のみで良いとする学校保健安全法施行規則に沿った形で本当に良いかどうか疑問を持たざるをえない。

学校保健安全法の改定後すでに5年が経過しているが，全国の大学でどのように対応しているか明らかでない。平成21年9月に開催された本協議会総会において立ち上がった「胸部検査のあり方を考える調査研究班」では本協議会会員校における定期健康診断時における胸部X線検査の現状を知り，今後のあり方を検討するためにアンケート調査を行った。本稿ではアンケート調査をもとに定期健康診断時の胸部X線検査のあり方を提言する。

方　法

全国国立大学法人等施設協議会会員校84校に対して全国国立大学法人等施設協議会事務局を介してアンケート調査依頼文およびアンケート内容をメール貼付で依頼し，原則的に大学名を匿名化しない状態で回答用紙をファックスもしくはメールで班長に回答する方式をとった。

アンケート調査内容の概要は以下のごとくである。

1）現有設備の有無と撮影装置について

[1]東北大学保健管理センター　[2]弘前大学保健管理センター　[3]大分大学保健管理センター　[4]岡山大学保健管理センター
[5]北海道大学保健管理センター　[6]千葉大学総合安全衛生管理機構
（著者連絡先）飛田　渉　東北大学保健管理センター　〒980-8576　仙台市青葉区川内41

2）定期健康診断の実施時期
3）定期健康診断時の胸部X線検査状況，検査装置および撮影対象学年
4）平成17年4月の学校保健法施行規則改定後の対応
5）1年時と2年時以上における疾病発見状況
等ついてである。

調査期間は平成21年11月6日より平成22年1月20日までとした。

結　果

アンケートに対する回答は75校より得られた（回答率89.3%）。

＜現有設備の有無と撮影装置について＞

大学に胸部X線撮影装置が備わっているところは28%の21校で，多くの大学では胸部X線撮影装置が備わっていなかった。現有設備の内訳については15校では直接撮影装置が設備されていたが6校は間接撮影装置のみのであった。また，デジタル装置が設備されている大学が7校にみられた。

＜定期健康診断の実施時期＞

定期健康診断の実施時期は4月に実施している大学が大部分であり，次に5月，6月と続いた。3月や10月に実施しているところも少ないながらみられた（図1）。

＜定期健康診断時の胸部X線検査状況および撮影対象学年＞

定期健康診断時の胸部X線検査はほとんどが学外装置で行っており（62校），学内装置のみで実施している大学は7校であった。他の6校は学内と学外装置の併用であった。しかも間接撮影で行っている大学が大部分で（62校），直接撮影のみが2校，間接撮影と直接撮影の併用が4校であった。

＜平成17年4月の学校保健法施行規則改定前後の対応＞

平成17年4月の学校保健法施行規則改訂後特に変更措置をとらなかった大学は53校（70.7%），で何らかの変更措置をとった大学が22校（29.3%）であった。

図2には変更措置をとらなかった53大学の対象学生を示す。44校（83%）は全学年を対象としていた。一方，図3に示すごとく，変更措置をとった22校では平成17年度においては20校が全学年対象であったが，平成21年度においては1年生のみに対象をしぼった大学はわずか1校で，1年生＋2年生以上の希望者，1年生＋卒年次，1年生＋希望者＋医療系学生，1年生＋

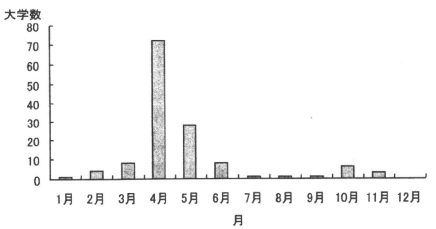


図1　平成21年度定期健康診断の時期（複数回答）

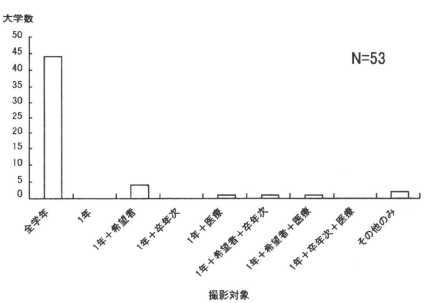


図2　学校保健法施行規則改訂後変更無しの大学における撮影対象

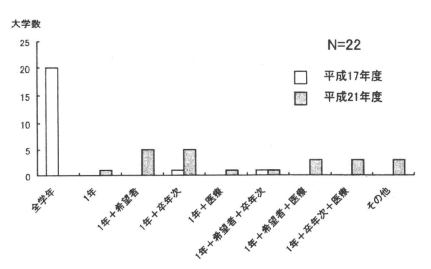


図3　学校保健法施行規則改訂後変更有りの大学における撮影対象

表1　回答が得られた75大学における定期健康診断受診者数と異常者数

		受診者数	異常所見者数	1000人当たり
平成19年	1年生	88,942	482	5.4
	2年生以上	223,700	925	4.1
平成20年	1年生	95,015	534	4.6
	2年生以上	232,363	1,028	4.4
平成21年	1年生	93,701	523	5.6
	2年生以上	224,932	872	3.9

卒年次＋医療系学生を対象にした大学がそれぞれ5校，5校，3校，3校であった。

1年生以外を撮影対象としている主な理由については，(1)就職活動（既に3年次から始まっている）を行う上で胸部X線検査結果が要求されるから（55校），(2)教育実習で胸部X線検査結果が要求されるから（47校），(3)新たな肺結核が2年生以上に発見されるから（31校），(4)非結核性肺疾患が2年生以上に発見されるから（28校）であった。(5)アルバイトを行う上で胸部X線検査結果が要求されるからを理由に挙げている大学も13校にみられた。

＜1年生と2年生以上における疾病発見状況等＞

表1は回答が得られた大学75校における過去3年間の定期健康診断受診者数と胸部X線検査による異常所見者数および1,000人当たりの異常所見者数である。1年生のみの異常所見者数は1,000人当たり5.4～5.6人であった。2年生以上における異常所見者数は1,000人当たり3.9～4.1であった。1年生における異常所見者数に比べ2年生以上における異常所見者数はやや少ないものの1,000人当たり4名前後であった。具体的な疾患としては疑いの例も含めて肺結核，自然気胸・ブラ，心疾患の順であった。肺腫瘍もチェックされたケースが見られた。2年生以上においても同様な傾向が見られた。

＜今後の対応＞

今後の対応について回答が得られた74校（1校は回答無し）についてみると変更無しが68校（92%），変更予定が6校（8%）であった。前述のごとく22校は既に変更しているので，変更していない53校についてみると変更無しが49校（92%）で変更予定が4校（8%）であった。全学年を対象としていた44校すべては今後も変更無しの回答であった。全回答校の59%は今後も引き続き全学年とする大学と思われた。

考　察

学生の健康診断は学校保健安全法によって全学生を対象に毎年実施しなければならない[3]。また学校保健安全法施行規則によって結核の有無を検討することが示されているものの，平成17年4月の学校保健安全法施行規則改訂を契機に，学生定期健康診断における結核の検査のための胸部X線検査の実施学年はそれまでの「大学の全学年」より「大学の第一学年」に変更された[4]。しかしながら，先行研究において木村らは既に定期健康診断時において発見された新入生と新入生以外の活動性肺結核罹患者の発生状況について全国の国立大学法人87校を対象に調査した結果を報告しており，①新入生よりも新入生以外での発見数が多かった，②留学生においては日本人学生よりも発見頻度が高かった，③関東・甲信越地区や近畿地区では学生10万人当たりの発生数が特に多く，次いで中国，九州，東海・北陸地区の順で，東北，四国，北海道地区では少なかったことを示した[5]。八田らも学生数4,000人以上の大学137校を対象として胸部X線検査と肺結核罹患者についてアンケート調査を行い，同様な結果が得られており[6]，胸部X線検査を全国一律に「大学の第一学年」のみとすることに警鐘を鳴らした。

また，今回の調査結果によると，多くの大学では全学部全学年を対象に健診時胸部X線検査を実施しており，今後も変更しない方針の大学が多かった。変更した大学の中で1年生のみとしたところはわずか1大学であり，他の大学では1年生に卒業年度，医療系学生，希望者などプラスアルファーのクラスターを加えた形で実施していた。1年生のみに限定していない理

由として就職活動のため，医療系の実習や教育実習のための理由を挙げていた大学は50％を越えていた。また，約40％の大学では2年生以上の学年において結核性およびブラ，気胸などの非結核性の肺疾患や心疾患などが発見されたことをあげていた。

従って，かかる先行研究や今回の調査結果に基づくと大学における定期健康診断時の胸部X線検査対象者の枠を広げる必要があると考えられた。

対象者枠を広げる際には大学の規模，学生の構成等各大学の実状に即して対応する必要があるが，本調査研究班ではアンケート調査をもとに胸部X線検査の対象者をできるだけ網羅する形でまとめることを考えた。

次のように，一年生以外の学生でも定期健診時胸部X線検査を省略できない場合がいくつかあげられよう。

臨床実習や教育実習を行う医療系学生や教育系学生の場合である。医学部，歯学部，薬学部の学生，看護学科の学生，臨床検査科や放射線検査科等医療系学生は早い学年より病院での臨床実習が組まれている。福祉・介護系学生も福祉介護施設での実習がある。また，教育実習は大方の大学では3，4年次の教育プログラムに組まれている。学生は肺結核に罹患すると多くの人への感染源となりうるデンジャラスグループであると考えられるので少なくとも臨床実習や教育実習等医療施設，福祉・介護施設あるいは学校においての実習に係る学生は毎年胸部X線検査を実施することは不可欠と思われる。労働安全衛生法[13]でも，学校，病院，診療所，助産所，介護老人保健施設又は特定の社会福祉施設において業務する場合には，胸部X線検査を省略できないことになっている。大学生もこれに準じた対応を考える必要がある。

前年度に異常を指摘されて経過観察のリストに載っている学生も省略することはできない。

また，現状においては殆どのケースにおいて就職活動およびアルバイト等のために企業側，アルバイト先側から健康診断証明書の提出が求められている。就職活動は卒業年次に関係なく行われており，大部分の大学では3年次から行われている。その際胸部X線写真の情報がないまま健康診断証明書に異常なしと記載して良いかどうかが問題となる。また，3年次，4年次において1年次の胸部X線検査結果を以て異常なしとするのも問題である。また大学生の活動の場は大学というキャンパス内には止まっていない。アルバイト等学外活動も行われており，一般社会人と生活の面では何ら変わらない。アルバイト等で学外での交流機会が多い学生も胸部X線写真撮影を含めて自分の健康チェックをしておくことは社会的な観点からも必要である。従って，就職活動やアルバイト等他の理由で胸部X線検査を希望する学生に対しては対象者に含めるべきである。

更に，アンケート調査結果からも推察されるように，約40％の大学では2年生以上の学年において結核性および非結核性の肺疾患などが発見されており，異常所見の頻度は1,000人当たり4人前後であった。頻度として決して高いとは思われないが，異常者の早期発見という健康診断の目的からすればこの数字は無視することはできない。太田らは2005年度に1年間で気胸と診断された例（175例）の約50％は定期健康診断時に発見されたと報告している[14]。この点からも日頃から自分の健康管理に注意し，定期健康診断を機会に胸部X線検査を希望する学生を受け入れるルートを希望枠として設けておく必要があろう。

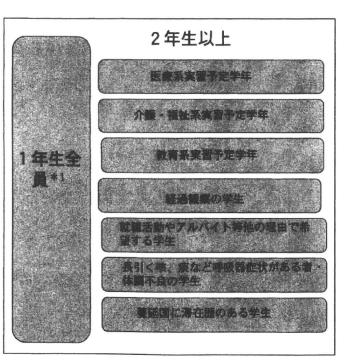
定期健康診断時あるいはその近い時期に，長引く咳や痰を訴える学生や体調不良学生は，ハイリスク者として胸部X線検査の対象者とすることも必要であろう。

大学のグローバル化が進み，肺結核が蔓延している国に滞在歴のある学生が増加しており，今後ますます増加することが予想される。従って，1年に1度の胸部X線検査により胸部の異常の有無をチェックすることは，留学生の多い大学や結核患者発生数の多い地域ではむしろ必要なことと思われる。帰国学生や外国からの留学生を含む蔓延国に滞在歴のある学生は，胸部X線検査対象クラスターに含むべきであると考える。しかしながら，帰国日本人学生の場合には胸部X線検査の必要性を理解してもらうことは容易と考えられるが，外国からの留学生の場合には社会的文化的環境の違いにより十分理解されない可能性があろう。その場合には外国語による説明書を作成するなどの工夫が必要と思われる。

現時点においては調査研究班としては図4の

ように，「1年生＋医学部，歯学部，薬学部の学生，看護学科の学生，臨床検査科や放射線検査科等の医療系実習予定学年＋介護福祉系実習予定学年＋教育系実習予定学年＋経過観察の学生＋就職活動やアルバイト等を含む希望する学生＋長引く咳や痰を訴える学生や体調不良学生＋蔓延国に滞在歴のある学生」とすることを提案したい。医療系学生がいない大学においては「1年生＋教育系実習予定学年＋経過観察の学生＋就職活動やアルバイト等を含む希望する学生＋長引く咳や痰を訴える学生や体調不良学生＋蔓延国に滞在歴のある学生」となる。一方，胸部X線検査をしない学生も出てくるので，そのような学生も含めて定期健康診断時以外でも長引く咳や痰や血痰などの呼吸器症状を伴った場合には，肺結核の有無のチェックのために，速やかに保健管理センターや最寄りの医療機関を受診するように啓発活動を行う必要がある。3年次などへの編入学生は新入生の枠で行えば良い。

学校保健安全法施行規則では，撮影装置は結核患者や結核発病のおそれがあると診断されている者以外はX線間接撮影を行うものとするとされている。しかしながらこの点についても大学の実状にあっておらず，一律にすることは問題と思われた。大学75校のうち学外のX線装置で健康診断を実施している大学が62校みられ間接撮影装置で実施していた。一方，学内装置単独もしくは学外装置との併用で実施している大学は13校であった。学内装置で健康診断を実施している大学では，胸部X線撮影を直接撮影で実施していた。一般的に被曝線量は直接撮影の方が少ないとされている。被曝線量の低減化をはかることは大切なことであり，直接撮影で実施している施設においてはあえて間接撮影に切り替える必要はない。



*1　3年次等へ編入した学生を含む

図4　本研究班として提案する定期健康診断時の胸部X線検査の対象学生

前述のごとく，健康診断そのものは学校保健安全法第13条では毎年実施するように定められているので，健康診断の結果を胸部X線検査無しに判断せざるを得ない学生も出てこよう。胸部X線検査をした学生，しない学生が混在することになり混乱を来す可能性がある。また，胸部X線検査をしない健康診断となると受検しない学生が多くなり，受診率の低下をきたすことも懸念される。従って，現実的には各大学の留学生の多寡も含めた学生の構成や地域性を考慮して，各大学の実状に即した対応を考えるべきであろう。なお，大学院生に対しては学部学生に準じて行えば良いと考える。

まとめ

国立大学法人保健管理施設協議会会員校84校のうち75校より回答が得られた。平成17年4月の学校保健法施行規則改定後，定期健康診断時の胸部X線検査の対象クラスターを変更したのは3割の大学で7割は変更しなかった。変更無しの大学の内83％の大学では対象学年を全学年としていた。変更した大学で対象学年を1年生のみとしたのは僅か1校のみで他は医療系学生，希望者，卒年次学生との組み合わせであった。大学の規模や学生の構成を考慮すると，定期健康診断時における胸部X線検査を全国一律に「大学の第一学年」とすることに問題があると思われた。本調査研究班としては学校保健安全法施行規則を踏まえて定期健康診断時の胸部X線検査の対象を「1年生＋医学部，歯学部，薬学部の学生，看護学科の学生，臨床検査科や放射線検査科等の医療系実習予定学年＋介護福祉系実習予定学年＋教育系実習予定学年＋経過観察の学生＋就職活動やアルバイト等を含む希望する学生＋長引く咳や痰を訴える学生や体調不良学生＋蔓延国に滞在歴のある学生」とすることを提案することが妥当と思われた。しかしながら，現実的には各大学の実状に即した

対応を考えるべきであろう。本調査結果が各大学において定期健康診断時の胸部X線検査のあり方を検討する上で参考になれば幸いである。

（本論文の概要は第48回全国大学保健管理研究集会に特別報告として発表された。）

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引用文献

1）Nohara T, Terao H, Tobe K, et al. Risks of chest X-ray examination for students. Acta. Med. Okayama 63:43-47, 2009

2）野原隆彦，寺尾英夫，戸部和夫，他．胸部X線撮影時の被ばく線量—第6報—　CAMPUS HEALTH 45（1）：233-235，2008

3）学校保健安全法
http://www.houko.com/00/01/S33/056.HTM

4）学校保健安全法施行規則
http://law.e-gov.go.jp/htmldata/S33/S33F03501000018.html

5）木村純子，野田惠子，楠田康子，他．新入生と新入生以外の活動性肺結核患者の発生状況から見た，学校保健法施行規則改定の問題点について　CAMPUS HEALTH 43（2）：77-82，2006

6）八田文裕子，岩本優美子，山下紀子，他．大学の学生定期健康診断に胸部X線検査は必要か？　CAMPUS HEALTH 47（2）：73-77，2010

7）国立大学保健管理センター所長会議　2．胸部X線検査　学生の健康白書1984　p7-11　1987

8）国立大学等保健管理施設協議会　3．胸部X線検査—未だに問題の肺結核　学生の健康白書1995　—応用研究編—　p24-27　1998

9）国立大学等保健管理施設協議会　5．肺結核　学生の健康白書2000　p63-73　2002

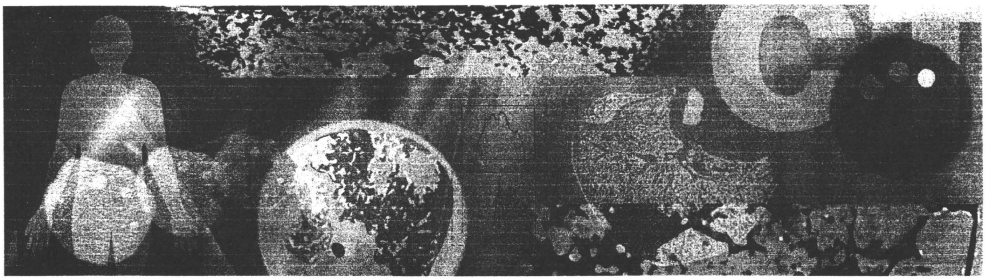
10）国立大学等保健管理施設協議会　5．胸部X線検査　学生の健康白書2005　p63-74　2008

11）長尾啓一．大学生と結核　CAMPUS HEALTH 45（2）：29-34，2008

12）飛田　渉．シンポジウム「学生の健康白書から見えてくるもの」2　胸部X線検査　肺結核，気胸　CAMPUS HEALTH 45（1）：62-63，2008

13）労働安全衛生法　www.mhlw.go.jp/new-info/kobetu/roudou/gyousei/anzen/1001-1.html

14）太田美智，三井栄子，長谷川洋子，他．大学生における気胸の発生状況　CAMPUS HEALTH，45（2）：159-164，2008



低線量肺がんCT検診の知識と実務

NPO法人 肺がんCT検診認定機構　監修
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Ohmsha

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A Training Session in a Clinical Simulation Laboratory for the Acquisition of Clinical Skills by Newly Recruited Medical Interns

Akinobu Yoshimura[1-3], Toshiro Shimura[1-3], Chol Kim[2], Gen Ishikawa[2], Shuji Haraguchi[4], Tadaaki Ohno[5], Hiroki Hayashi[5], Hiroshi Nakano[6], Takashi Nitta[3,6] and Masako Takaoka[6,7]

[1]Academic Quality and Development Office, Nippon Medical School
[2]Working Committee of the Clinical Simulation Laboratory, Nippon Medical School
[3]Board of Education, Nippon Medical School
[4]Department of Surgery, Nippon Medical School
[5]Department of Internal Medicine, Nippon Medical School
[6]Management Committee of Medical Interns of Nippon Medical School Hospital
[7]Nursing Department, Nippon Medical School Hospital

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—Report on Experiments and Clinical Cases—

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[5]Department of Internal Medicine, Nippon Medical School
[6]Management Committee of Medical Interns of Nippon Medical School Hospital
[7]Nursing Department, Nippon Medical School Hospital

Abstract

In organized orientation programs for newly recruited medical interns of the Nippon Medical School Hospital, the working committee of the clinical simulation laboratory introduced a laboratory training session that was designed to improve the clinical skills of the medical interns. The session consisted of 6 training courses, comprising internal examination, tracheal intubation, auscultation of heart sounds, bandaging and the collection of samples of venous and arterial blood. Medical interns rotated to a new course every 30 minutes and did practical trainings in each of the 6 skills. A total of 36 newly recruited medical interns participated in the training session. The majority of medical interns took part in the practical training actively and positively. The session was efficiently carried out from the standpoints of human resources and the teaching hours involved. A post training questionnaire survey, completed by the medical interns, revealed that many of them valued the sessions for comprehensibility of the instructions, the descriptions in the manual and the content of the training; however, only 21% thought that they had successfully acquired the clinical skills. Medical interns must continually engage in self-training to steadily acquire basic clinical skills. The convenience of a clinical simulation laboratory, together with the reinforcement of the education of clinical skills during internship, is necessary to strengthen the educational benefits of the training session.
(J Nippon Med Sch 2010; 77: 209–213)

Key words: simulation-based training, basic clinical skills, training session, newly recruited medical interns

Correspondence to Akinobu Yoshimura, Academic Quality and Development Office, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan
E-mail: ay1004@nms.ac.jp
Journal Website (http://www.nms.ac.jp/jnms/)

Introduction

A new training system for newly recruited clinical interns has been in place in Japan since 2004. At the same time, educational programs for medical interns have been provided by all teaching hospitals and university hospitals[1]. The programs showed that an objective of internship is to acquire clinical skills including airway management, cardio-pulmonary resuscitation, collections of blood samples from veins and arteries, lumbar puncture and tracheal intubation[1]. These training programs, which promote the acquisition of clinical skills, are attractive to medical interns. Furthermore, intern education is an important component of risk management, and this may be sufficient reason in itself for health service providers to continue to support this type of program[2]. Therefore, one might assume that initial clinical skills training for medical interns in the early stages of their internship would be of great benefit, from the view-points of motivation and risk management.

The transition from medical student to medical intern is a big cultural leap. The Nippon Medical School Hospital now manages the transition with organized orientation programs. Among these programs, a training session was introduced by the working committee of the clinical simulation laboratory to improve the clinical skills of newly recruited medical interns[3]. We describe here the technique that was used to provide this effective clinical skills training and the results of a post training questionnaire survey completed by the trainees, which aimed at assessing the value the interns placed on the program.

Subjects and Methods

In April 2009, as a part of the organized orientation programs for newly-recruited medical interns, a training session for clinical skills was implemented using a clinical simulation laboratory and 3 small-group learning rooms. The aim of the session was to train medical interns in basic clinical skills. Beforehand, all interns were required to read the training manual, in which the procedures of each training course were described and the specific behavioral objectives were clearly defined.

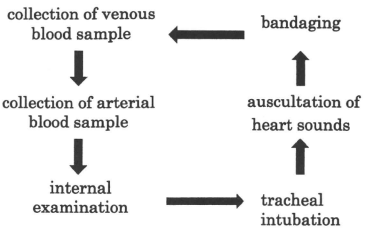

Fig. 1 Training Session for Clinical Skills
Medical interns moved in rotation every 30 minutes and took practical training in each of the 6 skills.

The session consisted of 6 training courses, comprising internal examination, tracheal intubation, auscultation of heart sounds, bandaging and collection of venous and arterial blood samples. Five simulators were used in the session, which included Gynecologic Simulator (S503 GYM/AID, Gaumard Scientific, Miami, USA), Airway Management Trainer (Laerdal Medical Japan K. K., Tokyo, Japan), Cardiology Patient Simulator "K" (Kyoto Kagaku Co., Ltd., Kyoto, Japan), Simulator Intravenous Arm II (M50-B, Kyoto Kagaku Co., Ltd.) and Arterial Puncture Wrist (M99, Kyoto Kagaku Co., Ltd.). Medical interns rotated to a training course, every 30 minutes and took practical training in each of the 6 skills **(Fig. 1, 2)**.

At the end of the training session, the medical interns were required to complete a 5-question survey using a 4-point scale (1=poor, 4=good). The 5 items in the questionnaire were as follows: question 1: "Were the instructions comprehensible?"; question 2: "Were the descriptions in the manual comprehensible?"; question 3: "To what extent did you acquire clinical skills?"; question 4: "To what extent were you content with the session?"; and question 5: "Will you use the clinical simulation laboratory for self-training?" **(Fig. 3)**.

After completing the training session, medical interns had free access to the clinical simulation laboratory and were encourage to engage in voluntary skills-training.

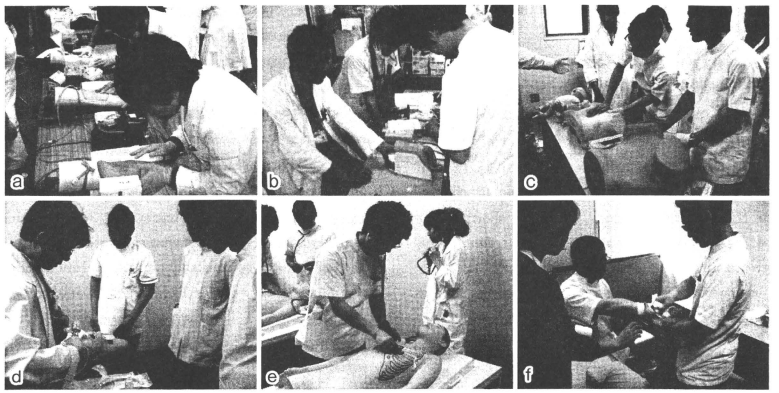

Fig. 2 Photographs of Training Sessions for Clinical Skills
a: collection of venous blood sample, **b**: collection of arterial blood sample, **c**: internal examination, **d**: tracheal intubation, **e**: auscultation (heart sounds). **f**: bandaging

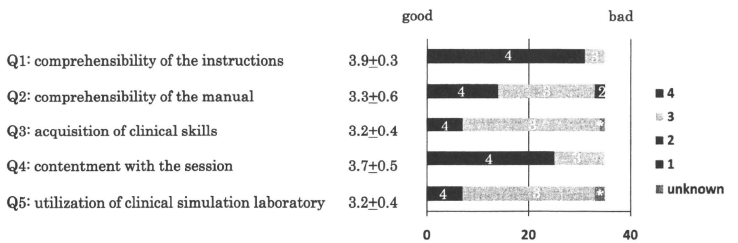

Fig. 3 Results of Post Training Questionnaire Survey
At the end of the training session, the medical interns were required to complete a questionnaire survey in which they answered five questions using a 4-point scale (1= poor, 4= good). *Asterisks mean no opinion.

Results

A total of 36 newly recruited medical interns of the Nippon Medical School Hospital participated in the training session. They were divided into 6 groups of 6 members. The sessions were all efficiently carried out from the standpoints of human resources and teaching hours. Seven physicians and 4 nurses were required as instructors. The entire session lasted 3 hours 30 minutes and consisted of 3 hours of training, 15 minutes of orientation preceding the first session, and a 15-minute rest period at the end of the third session.

The overall response rate of interns to the questionnaire survey provided at the end of the session was 97% (35 of 36 interns). **Figure 3** shows the result of the questionnaire survey. The mean values of question 1, 2, 3, 4, and 5 were 3.9, 3.3, 3.2, 3.7, and 3.2, respectively. Of the respondents, 89% (31 of 35) and 40% (14 of 35) thought that the explanations given by the instructors and the descriptions in the manual, respectively, were easily understandable. Although only 21% (7 of 34) of interns thought that they had successfully acquired clinical skills, 71% (25 of 35) were satisfied with the session. All 33 interns who answered question 5 indicated that they would use the clinical simulation

laboratory for self-training in the future to a varying degree.

A majority of medical interns actively participated in the practical trainings in a positive fashion (**Fig. 2**). We received much additional feedback from participants. The most frequent ad hoc remarks were as follows: "very appreciative of the opportunity to participate in the training course," "felt that the time periods were too short and/or that there was an imbalance in the training time between the 6 different skill sets," "usefulness for internship," and a request that "other skills, such as lumbar puncture and chest tube drainage, could be included in future programs."

Discussion

We have described a program for the efficient inductive training of clinical skills for newly recruited interns and presented the results of a post training questionnaire survey.

Simulation-based education is characterized by a safe environment, proactive and controlled training, trainee/team/system-centered education, feedback- and debriefing-based education and a reproducible, standardized objective[4]. In medical training, it is impossible ultimately to avoid encountering 'real-life' patients if the necessary skills of health professionals are to be acquired[4]. On the other hand, there is also a responsibility to provide appropriate treatment and to ensure patients' safety and well-being. Balancing these two needs represents a fundamental ethical dilemma in medical education. Simulation-based medical education can be a valuable tool for reducing these ethical and practical dilemmas[4]. Lynagh et al have demonstrated that clinical simulation laboratories lead to greater improvements in procedural skills compared with standard training or no training at all when assessed with simulator performance[5]. Therefore, initial clinical skills training for medical interns in the early stages of their internship must be both effective and valuable.

Because the change from medical student to medical intern is a big cultural leap for new recruits, organized medical orientation programs are indispensable for ensuring a trouble-free transition. Therefore, since 2008, we have conducted training sessions for clinical skills using a clinical simulation laboratory and 3 small-group learning rooms, as a component of our organized orientation programs.

The session consists of 6 training courses, comprising an internal examination, tracheal intubation, auscultation of heart sounds, bandaging, and the collection of samples of venous and arterial blood. We selected these basic skills to correspond with simulators in the clinical simulation laboratory and with the core clerkships in internal medicine of the Society of General Internal Medicine, the Clerkship Directors in Internal Medicine in the United States, and the training program for medical interns in the Nippon Medical School Hospital[1,6,7].

The session was efficiently carried out from the standpoints of both human resources and the teaching hours involved and, therefore, can be considered to contribute to the reduction of the overall teaching burden on instructors. Although many medical interns valued the sessions for comprehensibility of the instructions, the descriptions in the manual, and the content of the training, only 21% thought that they had successfully acquired clinical skills. It was understandable that medical interns pointed out the shortness or the imbalance of the training time period. However, we consider this designated time period to be reasonable taking account of the time restriction of the session and the concentration of medical interns.

Although medical interns were permitted to make free use of the clinical simulation laboratory after completing this session and were encouraged to continue to access the facility for voluntary skills-training, their subsequent utilization was disappointing. We have implemented consecutive training sessions, such as tracheal intubation, chest tube drainage, and lumbar puncture, to further promote additional clinical skills in medical interns. Taylor has suggested that undergraduate training in procedural skills is inadequate and has recommended comprehensive training programs and techniques for quality assurance to address this deficiency[8]. Consequently, we believe that mandatory supervision of key skills together with

opportunities to supplement the limited experience are needed during the intern year to ensure that all interns acquire a uniform standard of clinical experience[9].

Medical interns must continually engage in self-training to steadily acquire basic clinical skills. The convenience of a clinical simulation laboratory, together with the reinforcement of training in clinical skills during internship, is necessary to strengthen the educational benefits of the training session.

References

1. Training Program for medical interns in Nippon Medical School main hospital (2008), in Japanese.
2. Leeder SR: Preparing interns for practice in the 21st century. MJA 2007; 186 (Suppl): S6–S8.
3. Shimura T, Yoshimura A, Saito T, Aso R: Unique medical education programs at Nippon Medical School. J Nippon Med Sch 2008; 75: 196–201.
4. Ziv A, Wolpe PR, Small SD, Glick S: Simulation-based medical education: an ethical imperative. Acad Med 2003; 78: 783–788.
5. Lynagh M, Burton R, Sanson-Fisher R: A systematic review of medical skills laboratory training: where to from here? Med Educ 2007; 41: 879–887.
6. Bass EB, Fortin AH 4th, Morrison G, et al.: National survey of Clerkship Directors in Internal Medicine on the competencies that should be addressed in the medicine core clerkship. Am J Med 1997; 102: 564–571.
7. Goroll AH, Morrison G, Bass EB, et al.: Reforming the core clerkship in internal medicine: the SGIM/CDIM Project. Society of General Internal Medicine/Clerkship Directors in Internal Medicine. Ann Intern Med 2001; 134: 30–37.
8. Taylor DM: Undergraduate procedural skills training in Victoria: is it adequate? Med J Aust 1997; 166: 251–254.
9. Boots RJ, Egerton W, McKeering H, Winter H: They just don't get enough! Variable intern experience in bedside procedural skills. Intern Med J 2008; 1–6.

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Histone deacetylase inhibitor enhances sensitivity of non-small-cell lung cancer cells to 5-FU/S-1 via down-regulation of thymidylate synthase expression and up-regulation of $p21^{waf1/cip1}$ expression

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Department of Internal Medicine, Division of Pulmonary Medicine, Infectious Diseases and Oncology, Nippon Medical School, Tokyo, Japan

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It is desirable to find more appropriate therapeutic opportunities in non-small-cell lung cancer (NSCLC) due to the current poor prognosis of affected patients. Recently, several histone deacetylase (HDAC) inhibitors, including suberoylanilide hydroxamic acid (SAHA), have been reported to exhibit antitumor activities against NSCLC. S-1, a novel oral fluorouracil anticancer drug, has been developed for clinical use in the treatment of NSCLC in Japan. Using an MTT assay, we analyzed the growth-inhibitory effect of 5-fluorouracil (5-FU), S-1, and SAHA against three NSCLC cell lines, as well as the breast cancer cell line MCF7 which is known to be highly sensitive to 5-FU. Combined treatment with low-dose SAHA enhanced 5-FU- and S-1-mediated cytotoxicity and resulted in synergistic effects, especially in 5-FU-resistant cells. Both the mRNA and protein expression levels of thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), and orotate phosphoribosyltransferase (OPRT), which are associated with 5-FU sensitivity/response, were analyzed in the cells undergoing treatment. 5-Fluorouracil-resistant lung cancer cells displayed high expression of TS mRNA and protein. Suberoylanilide hydroxamic acid down-regulated TS mRNA and protein expression, as well as repressed the rapid induction of this factor during 5-FU treatment, in all examined cell types. We also examined the status of the Rb-E2F1 pathway, with SAHA up-regulating $p21^{waf1/dp1}$ expression via promoter histone acetylation; this, in turn, blocked the Rb-E2F1 pathway. We conclude that combination therapy with SAHA and S-1 in lung cancer may be promising due to its potential to overcome S-1 resistance via modulation of 5-FU/S-1 sensitivity-associated biomarker (TS) by HDAC inhibitor. (*Cancer Sci* 2010; 101: 1424–1430)

Even though a wide range of anticancer agents have been developed, many patients with advanced solid tumors still exhibit poor prognosis. With respect to treatment of advanced lung cancer, there are many anticancer agents in clinical use, such as cisplatin (CDDP), carboplatin (CBDCA), docetaxel, paclitaxel, vinorelbine, gemicitabine, 5-fluorouracil (5-FU) derivatives, camptotecin-11 (CPT-11), etc. A number of combination therapy regimens employing platinum compounds have proven to be effective and are widely applied in the initial treatment for inoperative non-small-cell lung cancer (NSCLC).[1,2] In addition, docetaxel, pemetrexed, and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors have been reported to be effective in the context of second-line chemotherapy for NSCLC.[3–5] However, the effect of these therapies on improving patient survival remains far from satisfactory at present.[1–5] It is consequently desirable to find more appropriate therapeutic opportunities for this disease.

Recently, molecular-targeted therapy in the treatment of cancer has made substantial progress. Histone deacetylase (HDAC) inhibitors have been shown to acetylate the nucleosomal histones of condensed chromatin, and cause the reactivation of genes silenced by hyperacetylated histones.[6] Histone deacetylase inhibitors, including suberoylanilide hydroxamic acid (SAHA), have demonstrated therapeutic benefit as monotherapy in hematologic, breast, and bladder malignancies, as well as mesothelioma and NSCLC, without evidence of severe adverse events.[7–10] We previously examined the sensitivity of 16 NSCLC cell lines to HDAC inhibitors via an MTT assay.[11] Histone deacetylase inhibitors displayed strong antitumor activity against a subgroup of the NSCLC cell lines, suggesting the need for the use of predictive markers to select patients receiving this treatment. We have also analyzed gene expression profiles in these cell lines using cDNA arrays to identify transcripts that correlate with sensitivity to HDAC inhibitor treatment.[11] We related the cytotoxic activity of particular agents to the corresponding expression pattern in each of the cell lines using a modified National Cancer Institute (NCI) program.[11] We found that nine genes can distinguish sensitive from resistant cell lines.[11] Histone deacetylase inhibitors may also be promising new agents for the treatment of NSCLC. Recently, it has been demonstrated that combinations of HDAC inhibitors with well-established chemotherapeutics can provide synergistic antitumor effects.[12–14]

S-1 (composed of tegafur, 2-chloro-2,4-dihydroxypyridine [CDHP], and potassium oxonate in the molar ratio 1:0.4:1[15]), which inhibits dihydropyrimidine dehydrogenase (DPD) activity, has been developed for cancer chemotherapy including the treatment of NSCLC (Taiho Pharmaceutical, Tokyo, Japan).[15] Many studies have demonstrated that intratumoral DPD activity affects resistance to 5-FU.[16–18] In particular, NSCLCs may be considered to be a tumor type that commonly displays a high level of DPD expression, which in turn is associated with decreased 5-FU activity.[19] S-1 mediates its antitumor effect via the blockage of DPD by CDHP.[15,17] Tegafur is a prodrug that generates 5-FU in the blood largely as a result of metabolism by cytochrome p450 enzymes in the liver. The active metabolite of 5-FU, FdUMP, forms a covalent complex with 5,10-methylenetrahydrofolate and thymidylate synthase (TS), resulting in inhibition of DNA synthesis.[20] It is well known that TS is frequently overexpressed in cancer cells compared with normal tissues, and that tumors with high TS expression are generally resistant to 5-FU.[14,15,17,21,22] Thymidylate synthase expression seems to be one of the key molecules connected with sensitivity to S-1, DPD blocking agent. In a clinical trial of S-1 in advanced

[1]To whom correspondence should be addressed. E-mail: agemma@nms.ac.jp

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NSCLC patients (not having received prior chemotherapy) carried out in Japan, the response rate was 22% with the median survival time being 10.2 months.[23] Furthermore, a phase II trial of S-1 + CDDP combination revealed a 47% response rate and acceptable safety profile for NSCLC patients.[24] Thus, combining other agents with S-1 therapy may be a promising strategy for NSCLC patients.

In this study, we examined the antitumor effects of 5-FU/S-1 in combination with SAHA against lung cancer cells using an MTT assay. Combined treatment with low dosage SAHA enhanced 5-FU/S-1 cytotoxicity, as well as provided a synergistic effect in 5-FU-resistant cells. Using Cox multivariate regression analysis, Nakano *et al.* reported that TS, orotate phosphoribosyltransferase (OPRT), and DPD status to be significant prognostic factors in respect to determining the survival of resected NSCLC patients that were post-operatively treated with a combination of uracil and ftorafur (UFT).[23] In our study, we investigated the status and modulation after treatment of these three molecules. The mechanism underlying this synergy seemed to be related to down-regulation of TS mRNA and protein by SAHA.

Materials and Methods

Cell lines. Three lung cancer cell lines and one breast cancer cell line were used in this study, as follows: A549, PC9, PC9/f14, and MCF7. The A549 and MCF7 cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). The PC9 cell line was obtained from IBL (Gunma, Japan). PC9/f14 (A. Gemma, K. Takenaka) is a highly metastatic subline of PC9 cells established at Nippon Medical School Medical School using an artificial metastasis method.[25]

Drugs and growth-inhibition assay. Suberoylanilide hydroxamic acid was purchased from Sigma. 5-Fluorouracil was provided by Kyowa Hakko (Tokyo, Japan). CDHP was provided by Taiho Pharmaceutical. They were dissolved in dimethyl sulfoxide (DMSO) for *in vitro* studies.

We used the colorimetric MTT assay to examine the activity of 5-FU monotherapy or SAHA monotherapy against all three lung cancer cell lines, as well as MCF7 cells which are known to be highly sensitive to 5-FU.[26] In addition, combination therapy against these lines was also examined. For these studies, 5-FU, 5-FU with SAHA, S-1 (5-FU + CDHP 1 μM), and S-1 with SAHA, were incubated with cells for 72 h. Cell suspensions (200 μL, 5×10^3 cells/mL) were seeded into 96-well microtiter plates and 10 μL of drug solution added, at various concentrations. After incubation for 72 h at 37°C, 20 μL of MTT solution (5 mg/mL in phosphate-buffered saline [PBS]) was added to each well and incubation then continued for a further 4 h at 37°C. The IC_{50} value was defined as the concentration of drug(s) needed for a 50% reduction in absorbance (560 nm) based on cell growth curves.

Real-time RT-PCR analysis. Real-time quantitative RT-PCR assessment of TS, DPD, and OPRT gene expression was performed using the AB1 Prism 7700 Sequence Detector system (Perkin Elmer/Applied Biosystems, Foster City, CA, USA). The PCR primers were designed using the Primer Express software program (Perkin Elmer/Applied Biosystems). The relevant oligonucleotide sequences were as follows:

TS, sense primer: tctggaagggtgttttggag, antisense primer: cctccactggaagccataaa; DPD, sense primer: atggaggagtgtctgggaca, antisense primer: ttgaggccagtgcagtagtc; OPRT, sense primer: tggcatcagtgaccttcaagccctcct, antisense primer: tagtgttttggaaactgttgaggtt; GAPDH, sense primer: tggtatcgtggaaggactcatgac, antisense primer: atgccagtgacttcccgttcagc.

Total RNA was extracted from cultured cells and reverse transcribed using the RevaTra Ace Kit, with a random hexamer being used as primer (Toyobo, Osaka, Japan). A portion of the resulting cDNA was used for quantitative PCR in a 25 μL final volume, together with the desired primers, and SYBR Green PCR Master Mix (Applied Biosystems). The initial thermal cycling conditions were 95°C for 10 min, as recommended by the manufacturer, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The level of gene expression was expressed as ratio of the relevant mRNA in a particular sample to the level of GAPDH mRNA in that sample. Each sample was measured at least three times.[25]

Western blot analysis. Western blot analysis was performed as previously described.[27] The blotted membranes were first incubated overnight at 4°C with primary antibodies specific for the following antigens. Antibodies to TS, DPD, and OPRT were provided by Taiho Pharmaceutical. Antibodies against E2F1, phosphorylated Rb, p53, p27, p16, acetylated H3, and acetylated H4 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against $p21^{waf1/cip1}$ and poly-(ADP-ribose) polymerase (PARP) were purchased from Biosource International (Camarillo, CA, USA) while those against β-actin was purchased from Cell Signaling Technology (Beverly, MA, USA). The membranes were then incubated with peroxidase-conjugated secondary antibodies and protein was detected with enhanced chemiluminescence (ECL) Western blotting detection reagents (Amersham, Buckinghamshire, UK).

Chromatin immunoprecipitation (ChIP) assay. The ChIP-IT Express kit (Active Motif, Carlsbad, CA, USA) was used to perform chromatin immunoprecipitation assays.[28] PC9/f14 cells were treated with 10 μM SAHA for 24 h. Cells were treated with 1% formaldehyde, followed by glycine stop-solution. Cells were then collected, centrifuged at 4°C, and resuspended in lysis buffer. After an additional 30 min of lysis and 10 strokes in a homogenizer, nuclei were collected by 10 min of centrifugation at 2400*g* and resuspended in shearing buffer.

Enzymatic shearing cocktail was added to digest DNA, facilitating preparation of DNA fragments ranging in size from 200 to 500 bp. After centrifugation, 10 μL of the supernatants (containing sheared chromatin) were utilized as assay input. Immunoprecipitation was carried out overnight at 4°C using an incubation mixture containing 10 μL sheared chromatin, protein G magnetic beads, and 3 μg of antibodies (acetylated H3, acetylated H4; Santa Cruz Biotechnology). After centrifugation, washing, and elution, the samples were reverse cross-linked and treated with RNase A and proteinase K. DNA (both immunoprecipitated samples and input) was recovered by phenol/chloroform extraction and ethanol precipitation and then analyzed by PCR.

The primers were designed as follows: $p21^{waf1/cip1}$ promoter p1 (285 bp), forward primer: 5′-ACCAACGCAGGCGAGGGACT-3′, reverse primer: 5′-CCGGCTCCACAAGGAACTGA-3′; $p21^{waf1/cip1}$ promoter p2 (291 bp), forward primer: 5′-CGTGGTGGTGGTGTGCTAGA-3′, reverse primer: 5′-CTGTCTGCACCTTCGCTCCT-3′.

The conditions used were 95°C for 5 min, 35 cycles at 96°C for 1 min, annealing at 60°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min.

Statistical analysis. Data are presented as mean ± SE of at least three independent experiments. Statistical analysis was performed using an unpaired Student's *t*-test. *P*-values <0.05 were considered statistically significant.

Results

Effect of 5-FU, S-1 compound (5-FU + CDHP), SAHA, and combination treatments on cell growth *in vitro*. The activity of 5-FU against a panel of lung cancer cell lines, as well as MCF7 cells, was determined by MTT assay (Fig. 1). Clinical trials with 5-FU/S-1 have previously shown that serum levels in treated patients reached 1–3 μM.[29] PC9 cells were moderately resistant to 5-FU, as compared to MCF7 cells (which had previously been reported to be sensitive to 5-FU).[26] A549 cells

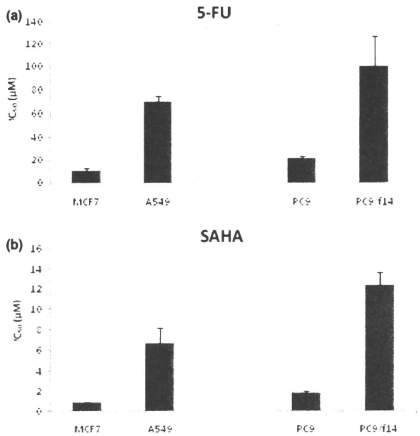

Fig. 1. Effect of 5-fluorouracil (5-FU) and suberoylanilide hydroxamic acid (SAHA) on cell growth *in vitro*. An MTT assay was used to investigate effects on cell viability mediated by 5-FU and SAHA. Cells were seeded into 96-well plates and treated with various doses of 5-FU and SAHA for 72 h, and then incubated with MTT reagent for 4 h. Cell viabilities were determined by measuring absorbance at 560 nm. The IC_{50} value was defined as the concentration of drug(s) needed for a 50% reduction in absorbance (560 nm) based on cell growth curves. Points, mean of at least three independent experiments; bars, SE. Note: The PC9/f14 cell line is a highly metastatic derivative of PC9 cells established at Nippon Medical School using an artificial metastasis method[26].

were also shown to be quite resistant to 5-FU (Fig. 1a). PC9/f14 cells were ~4.5-fold more resistant than their parental cell line, PC9 (Fig. 1a). Next, we examined the efficacy of SAHA against the same cell lines. Clinical trials with SAHA (Vorinostat) showed previously that serum levels in treated patients reached 0.43–2.98 μM.[7,8] In a similar vein to 5-FU response data, PC9/f14 and A549 cells were found to be resistant to SAHA, while MCF7 and PC9 were comparatively sensitive to this agent (Fig. 1b). For the purpose of determining a clinical setting for a SAHA and S-1 combination, we examined the growth-inhibitory potential offered by 5-FU monotherapy, S-1 compounds (5-FU + 1 μM CDHP) with or without low dose (1 μM) SAHA. One μM CDHP was within the serum concentration range found in clinical dosage within S-1-treated patients. Combination with SAHA significantly enhanced the growth-inhibitory effect of 5-FU, as compared to 5-FU monotherapy, in all examined cell lines. Similarly, S-1 compound (combination with CDHP) significantly enhanced the growth-inhibitory effect of 5-FU, as compared to 5-FU monotherapy, in PC9 and PC9/f14 cells ($P < 0.05$) (Fig. 2a). The combination of S-1 compounds (5-FU + CDHP) with SAHA significantly promoted the growth-inhibitory effect, as compared to S-1 compound (5-FU + CDHP) monotherapy, in A549 and PC9/f14 cells ($P < 0.05$) (Fig. 2a). These results indicated that the combination of S-1 and SAHA had a synergistic effect in certain cell types, especially in 5-FU-resistant cells. In PC9/f14 cells, each result was expressed as a ratio of MTT reduction in treated samples compared with the untreated sample (100%) for 5-FU alone or compared with the 1 μM SAHA-treated sample (92.4% and 87.0% of untreated samples [see Fig. S1]) for 5-FU therapy after SAHA 1 μM treatment, and concurrent therapy with SAHA 1 μM (see Fig. S1, Fig. 2b). The IC_{50} values of 5-FU therapy after SAHA 1 μM treatment and concurrent 5-FU therapy with SAHA 1 μM were 31.7 ± 8.42 μM and 30.4 ± 5.27 μM, whereas that of 5-FU monotherapy was 99.7 ± 26.7 μM.

These combination therapies significantly enhanced 5-FU sensitivity approximately threefold, as compared to 5-FU

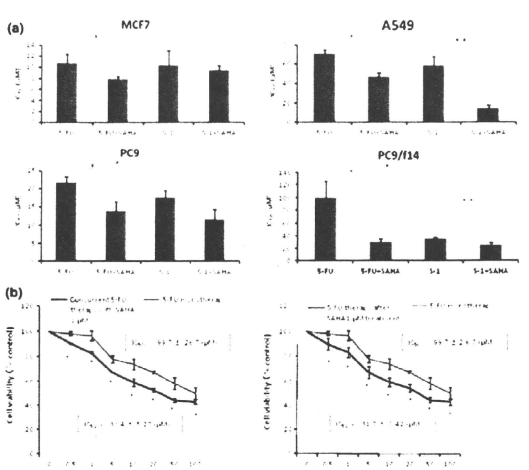

Fig. 2. Effect of combination therapy with 5-fluorouracil (5-FU)/S-1 and suberoylanilide hydroxamic acid (SAHA) on cell growth *in vitro*. (a) Indicated non-small-cell lung cancer (NSCLC) cells were treated with 5-FU or S-1, a compound composed of 5-FU and 2-chloro-2,4-dihydroxypyridine (CDHP) (1 μM), in combination with SAHA (1 μM). MTT assay was performed 72 h after addition of indicated drugs. IC_{50} was defined as the drug concentration required to inhibit cell proliferation by 50% compared with that of untreated control cells. Data represent the mean value of IC_{50} ± SE of three independent experiments in triplicate. *$P < 0.05$ compared to 5-FU alone. **$P < 0.05$ compared to S-1 alone. (b) 5-Fluorouracil therapy for 72 h after low-dose SAHA treatment (1 μM) for 24 h, and concurrent therapy for 72 h, was examined in PC9/f14 cells. Each result is expressed as cell viability in treated samples compared with the untreated sample (100%) for 5-FU alone or compared with the 1 μM SAHA-treated sample (92.4% and 87.0% of the untreated sample; Fig. S1) for 5-FU therapy after 1 μM SAHA treatment, and concurrent therapy with 1 μM SAHA treatment. Data represent the mean value of cell viability ± SE of three independent experiments in triplicate; *$P < 0.05$ compared to 5-FU monotherapy.

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monotherapy, in PC9/f14 5-FU-resistant cells (Fig. 2b). These results indicate that the combination of 5-FU with SAHA could have a synergistic growth-inhibitory effect against PC9/f14 cells.

5-Fluorouracil-resistant lung cancer cells display high levels of TS mRNA and protein expression. The expression level of TS, OPRT, and DPD mRNA in the lung cancer cell lines and MCF7 cells was quantified by real-time RT-PCR analysis (Fig. 3a). Thymidylate synthase mRNA expression in PC9/f14 cells, which are 5-FU resistant, was about 45 times the level of that seen with MCF7 cells, which are 5-FU sensitive. MCF7 cells have wild-type p53 status, while PC9 and PC9/f14 cells contain mutated p53[30,31] (Fig. 3b-1). By western blot analysis, the expression of TS protein also increased inversely in relationship to 5-FU sensitivity (Fig. 3b). Expression of OPRT and DPD protein did not correlate with 5-FU sensitivity (Fig. 3b).

Suberoylanilide hydroxamic acid down-regulates TS mRNA and protein expression in lung cancer cells. We examined the levels of TS, OPRT, and DPD mRNA and protein expression following SAHA treatment of PC9/f14 cells in order to identify key mechanism(s) underlying the synergistic growth-inhibitory effect mediated by combination of S-1 compounds + SAHA.

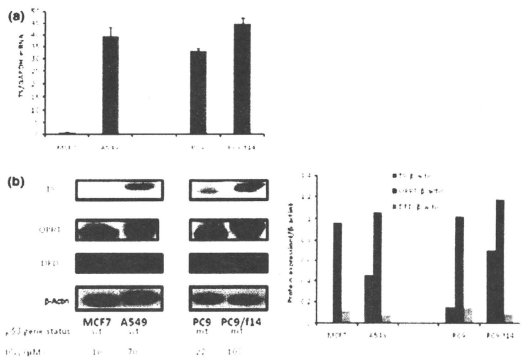

Fig. 3. Correlation of 5-fluorouracil (5-FU) sensitivity with expression of factors related to 5-FU metabolism in cancer cell lines. The expression of thymidylate synthase (TS), orotate phosphoribosyltransferase (OPRT), and dihydropyrimidine dehydrogenase (DPD) mRNA and protein was determined by real-time RT-PCR and western blot analysis, respectively, in lung cancer cell lines, as well as MCF7 cells. (a) The level of gene expression was expressed as ratio of the relevant mRNA in a particular sample to the level of GAPDH mRNA in that sample. The ratio was compared to the corresponding expression level observed in MCF7 cells. Points, mean of at least three independent experiments; bars, SE. (b) Expression of TS, OPRT, and DPD proteins were determined by western blot analyses. Quantitative analyses of protein expression ([TS, OPRT, and DPD]/β-actin) was examined using NIH image software.

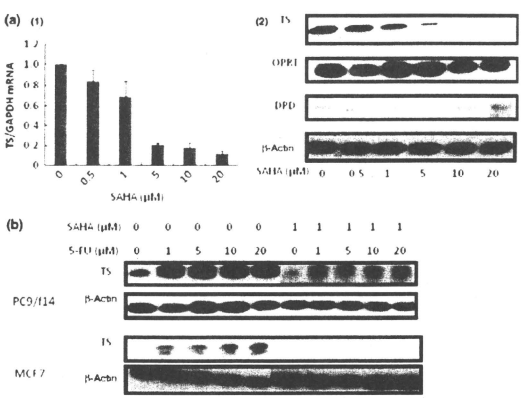

Fig. 4. Effect of suberoylanilide hydroxamic acid (SAHA) treatment on expression of the factors related to 5-fluorouracil (5-FU) metabolism and the expression of thymidylate synthase (TS) in cancer cell lines after 5-FU monotherapy and SAHA combination therapy. (a) The effect of SAHA treatment for 24 h on TS, orotate phosphoribosyltransferase (OPRT), and dihydropyrimidine dehydrogenase (DPD) mRNA and protein expression in PC9/f14 cells was examined by real-time RT-PCR and western blot analyses, respectively. (1) For the different doses of SAHA used, the level of gene expression was expressed as a ratio of the relevant mRNA to the level of GAPDH mRNA. Points, mean of at least three independent experiments; bars, SE. (2) Expression of TS, OPRT, and DPD proteins, following exposure to different doses of SAHA, were determined by western blot analyses. (b) PC9/f14 and MCF7 cells were incubated with various doses of 5-FU and SAHA (1 μM) for 24 h. Thymidylate synthase expression was determined by western blot analysis. A rapid increase in TS protein expression was observed after the exposure to 5-FU. Suberoylanilide hydroxamic acid suppressed 5-FU-mediated induction of TS protein.