とが望ましい．気管・気管支への腫瘍浸潤による喀血や圧迫による無気肺，癌性リンパ管症による呼吸困難などの緊急症状に注意する必要がある．

胸腔内への転移のなかでは，肺実質への転移よりも胸膜転移の方が一般的である．悪性細胞を伴う胸水の貯留により診断されることが多いが，CTやFDG-PETなどの画像診断で胸膜転移が疑われ，胸腔鏡により診断されることもある．胸水の増加による呼吸困難がおもな症状であるが，胸腔内留置カテーテルより1,000～1,500 ml/日程度廃液することにより症状の改善が得られる．胸水の貯留速度が緩徐であれば，廃液直後にマイトマイシンC10 mgを胸腔内に注入し，壁側と臓側の胸膜の癒着を促進して胸水貯留を抑制することは比較的簡便であるが，効果は部分的であることが多い．胸腔内へのシスプラチンなどの抗腫瘍薬の注入は，効果が明らかでなく一般に行われていない．最近，Chiらは初回手術における腫瘍縮小術の一部として胸腔鏡による胸膜播種巣の切除を積極的に試みているが，その効果はまだ確認されていない．やはり胸水のコントロールは他の臓器への転移と同様，有効な化学療法の有無がカギとなる．

4. 臍・皮膚（胸腹壁）転移

卵巣癌の臍転移や胸腹壁の皮膚転移はまれであるが，遭遇する．ラパロスコープやドレーンの刺入部位や開腹創への転移，腹膜播種巣からの直接浸潤のほか，皮下のリンパ路を経て，リンパ管の多い臍周囲やリンパ経路である胸腹壁の皮下に転移するものと考えられる．武下らは，転移性臍癌67例中9例（13%）が卵巣癌原発であったとしている[11]．他臓器への転移や腹腔内病変がなければ積極的な摘出術と化学療法の追加が推奨される．皮膚転移の場合は局所麻酔でも複数個の腫瘍摘出が可能である．また，シスプラチンやパクリタキセルは放射線増感効果を持つといわれており，化学療法と局所的な放射線療法の併用も効果が期待される[6]．

5. 骨転移

悪性腫瘍の骨転移は，臨床的に単純X線写真，骨シンチグラム，CT，MRIや最近ではFDG-PETを駆使した総合的画像診断によりかなり小さい転移巣まで診断可能になってきた．また，骨生検や吸引細胞診などが普及し，病理学的に原発巣や組織学的な診断も可能である．婦人科癌からの骨転移は比較的まれであるが，剖検例を対象にすると23.3%の症例に認められたとする報告がある[12]．しかし，それらの多くは子宮頸癌や子宮体癌であり，卵巣癌の骨転移は直接浸潤を除くと非常にまれであると考えられる．卵巣癌の遠隔転移例の検討では50例中2例（2.5%）[1]，88例中3例（3.4%）[2]のみが骨転移であったと報告されている．部位は胸椎や鎖骨で，疼痛を訴える場合が多く，骨転移の診断からの予後は4カ月と不良であるため[2]，疼痛除去のための対症的放射線療法が行われている．切除術や化学療法の有効性に関する報告は見当たらない．

6. 心嚢転移

心嚢転移は比較的まれで，癌性心膜炎による心嚢液貯留によって呼吸苦や胸部圧迫感を生じる．胸部X線像や心エコー検査により診断は容易であるが，心タンポナーデを発症すると致死的となることが多い．まず輸液を行い，心虚脱を改善するとともに，カテコラミンを用いて血圧を維持する．次いで，心嚢穿刺によりカテーテルを留置し廃液するとともに，マイトマイシンCを10 mgかOK432を10KE注入し，心膜癒着を試みることが推奨される．心嚢穿刺に際しては，十分なインフォームド・コンセントを得たうえで，循環器科専門医による実施が望ましい．心嚢転移後の予後は2.3カ月ときわめて予後不良[2]であり，心嚢内への薬物注入による有意な延命効果は期待できないとの報告が多いが，心嚢液再貯留の予防には有効であり，短期

的な延命効果は期待できそうである[13]

7. 脳・硬膜転移

脳転移は卵巣癌の2%程度にみられ，神経症状の発現によりCTで診断されることがほとんどである．転移後の予後は1.3カ月と転移部位別にみるともっとも予後不良である[2]．症状や部位にもよるであろうが，まず，切除術は推奨できない．一般的には全脳照射が行われ，化学療法の効果は少ないとされている．しかし，卵巣癌の診断後7年間に8レジメンの化学療法が行われ，多くの抗腫瘍薬に耐性と考えられた硬膜転移例において，50 Gyの全脳照射と，これに続くシスプラチン・パクリタキセルの静脈内投与とエトポシドの経口投与を用いた化学療法により完全寛解したとの報告[14]がある．放射線照射でblood-brain barrierがダメージを受けることにより，抗腫瘍薬の脳内移行が促進されることが推察されている[14]．

おわりに

卵巣癌の遠隔転移67例中，腹腔内病巣を伴わないものは13例のみであった[2]と報告されているように，外科的切除の対象となる遠隔転移はまれである．しかし，数少ない症例であっても，本稿で述べたような取り扱い方で転移部位別に対症的あるいは根治的に治療を続行することは可能であり，少なからず患者の予後やQOLの改善に役立つものと思われる．

卵巣癌の遠隔転移と診断されても，手術療法・化学療法・放射線療法などを組み合わせた集学的治療により完全寛解を得られる症例もあることを念頭におき，卵巣癌の治療にあたりたいと考える．

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特集　卵巣癌―エビデンスに基づく治療―

卵巣癌の組織型と化学療法

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上皮性卵巣癌において漿液性腺癌は欧米では90%以上を占めるものの，わが国では50%に満たず，一方標準的化学療法に抵抗性の明細胞腺癌は10～20%も占めている。したがって本邦においては上皮性卵巣癌の組織型に基づく化学療法の個別化の必要性は，欧米より高いと考えられる。すでに従来のCAP療法や現在標準とされているTJ療法の，明細胞腺癌や粘液性腺癌に対する有効性は低いと報告されており，これらの組織型にはイリノテカンやエトポシドとシスプラチンとの併用がむしろ有効であると報告されている。TJ療法は漿液性腺癌と類内膜腺癌に限って選択されるべきであろう。

はじめに

近年の上皮性卵巣癌に対する画一的な化学療法による予後の改善は限界に達したと考えるべきである。今後は組織型，分化度，残存腫瘍径，前治療の内容さらには薬剤耐性遺伝子など種々の予後因子により，患者ごと個別化した化学療法を選択しなければさらなる予後の改善は得られないと考えられる。1979年にEhrlichら[1]が報告して以来，シクロフォスファミド(CPA)，アドリアマイシン（ADR）およびシスプラチン（CDDP）との併用療法であるCAP療法が十数年間，初回寛解導入（1次）化学療法（first line chemotherapy）として用いられてきた。そしてCAP療法は上皮性卵巣癌のすべての病理組織型に適応され，とくに漿液性腺癌や類内膜腺癌に高い奏効率を示し，漿液性腺癌がほとんどを占める欧米において，もっとも信頼できるレジメンであったと考えられる。さらにその十数年間にはより高い奏効率とより良好な予後を求めて，CDDPを中心としたdose intensity（DI）を重視した[2]，大量化学療法が注目された時期もあった。しかし適応症例を選択する必要のある末梢血幹細胞移植（PBSCT）併用大量化学療法も含めて，DIには限界のあることがすでに報告されている[3][4]。

上皮性卵巣癌に対するfirst line chemotherapyは，1996年にMcGuire[5]によりパクリタキセル（PTX）とCDDPとの併用療法（TP療法）が，CPAとCDDPとの併用療法（CP療法）と比べてより高い奏効率と良好な予後を得たと報告されて以来，TP療法次いでPTXとカルボプラチン（CBDCA）との併用療法（TJ療法）が，CAP療法やCP療法に取って代

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わり，上皮性卵巣癌に対する標準化学療法とされるようになった。しかしたとえfirst line chemotherapyの奏効率が高まり，予後が改善されたといっても，5年生存率でみると依然として，進行期卵巣癌患者では約30%程度，上皮性卵巣癌患者全体でも約50%程度にとどまっている。しかも本邦では，上皮性卵巣癌に占める漿液性腺癌の割合が減少してきており，逆にCDDP耐性が問題とされている明細胞腺癌の割合が増加傾向にある[6]と報告されている。明細胞腺癌と同様，粘液性腺癌においてもkey drugであるCDDPに対する感受性の低下いわゆる耐性（自然耐性あるいは獲得耐性）が問題化されており，その機序の解明と克服について検討がなされている[7][8]。ただし欧米では卵巣癌の組織型において漿液性腺癌がほとんどを占めることから，化学療法の個別化に関する報告はほとんど見当たらないのが現状である。摘出組織あるいは細胞を用いた薬剤感受性試験を応用した化学療法の個別化の有効性を報告したものが散見されるのみである。卵巣癌患者の予後をさらに改善するもっとも近道的な手段は，各種予後因子による患者の個別化を行い，これに準じた化学療法をそれぞれ選択することであろうと考える。したがって本稿では本邦を中心とする限られたEBMに基づき，卵巣癌の組織型に基づく化学療法の個別化の可能性について述べることとする。

I. 漿液性腺癌・類内膜腺癌

本邦では初回手術時の病理組織学的診断において，漿液性腺癌がもっとも多く，上皮性卵巣癌の35～40%を占める[6][9]。CAP療法，CP療法，TP療法を中心とするプラチナ製剤を含む化学療法により72.5%と良好な奏効率が得られている。したがって漿液性腺癌と類内膜腺癌に対する化学療法のレジメンの選択は上記のようなものとなるが，どのレジメンが最良であるかの結論はまだでていないと考えられる。すなわち表1に示すように，欧米での上皮性卵巣癌に対する化学療法の変遷は1979年よりCAP療法，CP療法，TP療法，TJ療法・DJ療法と流れてきているが，奏効率という観点から判断する限り，CAP療法とCP療法，TJ療法とCAP療法あるいはCP療法，TJ療法とDJ療法のそれぞれの優劣は結論をだすに至っていないと考えられる。さらに各レジメンの副作用の程度やコスト面も考慮に入れると，包括医療制度が導入された本邦においては今後上記レジメンのうちどれがベストであるのか再検討を求められる時期が来るかもしれない。

またわれわれ臨床医が，病棟で直面する再発卵巣癌の病理組織型のほとんどは，漿液性腺癌である。他の組織型と比べて化学療法に対する奏効度は良好で，短期予後は改善されたと思われるが，5年生存率以後の長期予後に関しては予後不良といわれる明細胞腺癌と比較してそれほど良好とはいえない。杉山らの報告[9]では表2に示すように，漿液性腺癌のIII期とIV期の3年生存率は，それぞれ54.1%と35.7%，5年生存率はそれぞれ34.1%と4.0%で，一方明細胞腺癌のIII期とIV期の3年生存率は，それぞれ23.5%と11.4%，5年生存率もそれぞれ23.5%と11.4%でIII・IV期全体の5年生存率の差は10%以下であった。また筆者ら[6]の検討でもこの差は5%程度となっている。したがって化学療法に奏効すると認識されている漿液性腺癌や類内膜腺癌においても，組織型以外の薬剤感受性や長期予後に関与する因子を同定し，化学療法のさらなる個別化が必要と考えられが，この問題を言及する論文は見当たらない。

II. 明細胞腺癌

卵巣明細胞腺癌が化学療法に抵抗性であることは従来より報告[10][11]されていたが，欧米における発生頻度は5%前後で早期癌が多いため，初回手術による完全摘出がなされ，予後不良であることの問題性が低かったと考えられる。しかし本邦では近年，明細胞腺癌の発生頻度がと

表 1 上皮性卵巣癌に対する化学療法の変遷

1979	Ehrlich：CAP 療法の確立
1986	Omura（GOG 47）：CAP＞CA
1989	Omura（GOG 52）：CAP＝CP
1991	Ovarian Cancer Meta-Analysis Project：CAP＞CP
→	CAP vs CP の結論は不明のまま
1996	McGuire（GOG 111）：TP＞CP
1999	Ozols（GOG 158）：TJ＝TP（TJ の副作用少ない）
→	TJ vs CAP/CP の結論は？ (1999-JGOG で検討中)
2000	Columbo（ICON3）：TJ＝J alone/CAP（2 年生存率まで）
2001	Vasey（SCOTROC）：TJ＝DJ (TJ の神経毒性 vs DJ の血液毒性)

表 2 漿液性腺癌と明細胞腺癌の臨床進行期別予後

臨床進行期	漿液性腺癌			明細胞腺癌		
	患者数	3 生率(%)	5 生率(%)	患者数	3 生率(%)	5 生率(%)
IA-B	17	100	91.7	11	100	100
IC	22	80.4	80.4	38	76.8	60.1
II	13	83.1	72.7	10	88.9	88.9
III	145	54.1	34.1	31	23.5	23.5
IV	38	35.7	4.0	11	11.4	11.4
全体	235	58.6	44.1	101	57.9	52.0

（文献 9 より改変）

くに都市部において増加傾向にあり，上皮性卵巣癌の 20%以上を占めるようになったともいわれている。頻度の上昇とともに化学療法に抵抗性であることが大きな問題となりつつあり，術前の組織学的推定，手術完遂度の上昇および適切な化学療法の確立が求められている[10]。

明細胞腺癌に対する化学療法の後方視的な検討では，表 3 に示すように CAP・CP 療法の奏効率は高々22%（23 例中 5 例）であり，そのうち無病生存しているのは 2 例のみで，CAP・CP 療法に対して感受性はほとんどないと考えられた。そこで当科では 1996 年より明細胞腺癌に対する 1 st-line としてエトポシド(E)を用いた EP・EJ 療法を導入し，7 例に施行したが奏効例は得られなかった。症例数が少なく，1 st-line としての EP・EJ 療法が無効であると結論づけるのは時期尚早ではあるが，あまり期待できるレジメンではなさそうである。しかし，表 4 に示すように 2 nd-line 以後に選択した場合は，奏効率 29%で，CAP・CP 療法により何らかの修飾を受けたと思われる残存腫瘍に対して，EP・EJ 療法は試みる価値のあるレジメンと考えられる。一方，塩酸イリノテカン(CPT-11)と CDDP を用いた CPT-P 療法は，2 nd-line 以後に選択した場合，5 例中 2 例（40%）に PR が得られ，症例数は少ないものの奏効率からみると，もっとも期待できるレジメンであると考えられ，当科では 1998 年より明細胞腺癌に対する 1 st-line のレジメンとして導入している[12]。その後の検討で，明細胞腺癌に対する 2 nd-line として 8 例中 4 例（50%）に有効で，1 st-line としては 9 例中 5 例（56%）に奏効し

表 3　明細胞腺癌に対する初回化学療法のレジメン別奏効率

レジメン	症例数	評価可能例数	奏効度					奏効率(%)
			CR	PR	NC	PD	NE	
CAP・CP	44	23	2	3	2	16	11	22
EP・EJ	7	5	0	0	2	3	2	0
ActFtCY	2	1	0	1	0	0	1	100
合　計	53	29	2	4	4	19	24	21

(文献 10 より引用)

表 4　明細胞腺癌に対する 2 nd, 3 rd-line 化学療法のレジメン別奏効率

レジメン	投与例	評価可能例	奏効度					奏効率(%)
			CR	PR	NC	PD	NE	
EP・EJ	7	7	1	1	1	4	0	29
CPT+P	6	5	0	2	0	3	1	40
TXL+P	2	1	0	0	0	1	1	0
合　計	15	13	1	3	1	8	2	31

(文献 10 より引用)

たことがわかっている。一方，TJ 療法の効果は Enomoto らが 2003 年の米国臨床腫瘍学会で上皮性卵巣癌 84 例について報告したように，漿液性腺癌には 81%，類内膜腺癌には 71%有効であったのに対し，粘液性腺癌には 7 例中 1 例（14%），明細胞腺癌には 9 例中 2 例（22%）しか奏効しなかった[13]。また Sugiyama らの報告[9]でも，プラチナ製剤を含む化学療法に対して漿液性腺癌は 109 例中 79 例（72.5%）が奏効したのに対し，明細胞腺癌は 27 例中 3 例（11.1%）にしか効果が認められなかったとしている。欧米と比べ上皮性卵巣癌における明細胞腺癌の占有率が有意に高い本邦において，さらに奏効度や予後の良好な化学療法のレジメンが確立され，世界に発信されることが望まれる。

III. 粘液性腺癌

粘液性腺癌は欧米では上皮性卵巣癌の 10%程度で，本邦では約 20%を占めるといわれるが，その 2/3 は早期癌で完全摘出可能である[6]。粘液性腺癌に注目した化学療法についての報

表 5　病理組織型別化学療法の選択

病理組織型	1 st line	2 nd line	salvage
漿液性腺癌	TJ	CPT-P	?
類内膜腺癌	TJ	CPT-P	?
粘液性腺癌	EP	CPT-MMC	?
明細胞腺癌	CPT-P	EP	?

(文献 16 より改変)

告はほとんどない。薬剤感受性試験の結果から，Shimizu らは粘液性腺癌細胞株に対して CPT-11 がもっとも感受性が良好で，次いでマイトマイシン C（MMC）や ADR であったと報告し，CPT-MMC レジメンの臨床応用を推奨している[14]。粘液性腺癌は初回手術後の残存腫瘍径が 2 cm 未満であれば 8 例中 6 例（75%）に CAP 療法が有効であったが，残存腫瘍径が 2 cm 以上の場合は 6 例中効果を示したものは全くなく，さらに III・IV 期の 5 年生存率は 10%程度であったと筆者らは報告[6]している。粘液性腺癌の再発例に対し，有効な化学療法の確立は未だ報告されておらず，エトポシドとプラチナ製剤との併用療法（EP・EJ 療法）が 3 例中 1 例に PR を示したとの報告[15][16]

があるくらいである。first-lineの化学療法として粘液性腺癌に推奨されるレジメンは現在のところ見当たらない。前述したようにCAP療法やTJ療法に期待することには無理があり，CPT-11とMMCの併用療法[14]も紹介はされているがエビデンスに乏しい。

IV. 小細胞癌・扁平上皮癌

Youngらは卵巣の小細胞癌150例について臨床病理学的に報告しているが，IA期以外では化学療法と放射線療法を行ったIIB期の1例を除き全例が死亡しており，有効な化学療法は示されていない[17]。しかし胚細胞腫瘍に有効なブレオマイシン（BLM）を用いたBEP療法にCPA，ADR，ビンブラスチン（VBL）を併用する強力な化学療法が卵巣の小細胞癌に有効であったとの報告[18]がある。卵巣癌においては非常にまれな組織型であるため，有効な化学療法に関する報告が少なく，現時点では肺癌の小細胞癌に対するEP療法やCPT-P療法[19]，CPAとADRおよびビンクリスチン（VCR）をもちいたCAV療法[20]などのレジメンを応用することが奨められる。

卵巣の扁平上皮癌に対しては，化学療法だけでなく放射線療法も有効とはいえない[21][22]。最近では，卵巣類皮嚢腫より発生し腹腔内播種を示した扁平上皮癌に対し，積極的な腫瘍摘出術と術後TP療法により長期予後が得られたとの報告[23]がある。

いずれにせよ卵巣の小細胞癌や扁平上皮癌はその頻度の低さから，有効な化学療法を推奨するにはエビデンスが少ない。

おわりに

上皮性卵巣癌の組織型に基づく化学療法の個別化の可能性について述べた。表5に当科で行っている化学療法の選択基準を示す。これにより少なからず上皮性卵巣癌の予後は改善されるであろうが，その程度は決して満足のいくものではないであろう。今後は，化学療法に良好な感受性を示す漿液性腺癌と類内膜腺癌における再発後の薬剤耐性例に対する有効な化学療法の開発およびこの2種以外の組織型に対するより有効な化学療法の確立が大きな問題である。この問題を克服することにより上皮性卵巣癌の予後は大きく改善されるものと思われる。そのためには卵巣癌の化学療法の個別化は，組織型のみにとらわれていてはならず，組織型を超えた個々の卵巣癌患者の薬剤感受性に基づく個別化が必要となるであろう。これまでもMTT法，HDRA法，ATP-TCA法[24]など種々の薬剤感受性試験法が開発され，その臨床的有用性が報告されている。またマイクロアレイ法やCGH法を用いた薬剤耐性関連遺伝子の検索[25]も可能となった。卵巣癌患者に対し，化学療法の有効性と現在の限界をさらに詳しく説明することにより，上記方法を活用した化学療法の個別化が臨床的にさらに可能となると考えられる。

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DRUG DEVELOPMENT RESEARCH 62:124–133 (2004)

DDR

Research Commentary

Application of Expression Genomics in Drug Development and Genomic Medicine

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Expressed sequence tags and genome sequencing have virtually uncovered all the genes and transcripts in the human genome. Functional genomic analysis of these genes will undoubtedly reveal their physiological roles in cells in the near future. Coupled with the advent of DNA microarray, cellular response to perturbation can now be examined at genome-wide levels in a single analysis, yielding clues to the network of pathways and interacting pathways that underlie a comprehensive systems response to exposure to small molecule perturbants. This post-genomic information will be the foundation for knowledge-based in silico systems biology. It is envisioned that querying such relational databases will generate testable hypotheses as well as revealing information on networks of regulatory genes and pathways that could further fuel and shape molecular and drug target discovery, and ushering in a new era in genomic medicine. In this review, we will first discuss examples of laboratory results generated from high-throughput microarray analyses that illuminated previously unrecognized networks of regulatory genes and pathways that point to the mechanisms of action for the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate, and the green tea polyphenol, *epi*-gallocatechin gallate. We will then focus on the application of gene expression profiles in genomic medicine for predicting treatment response in cancer, using clinical specimens obtained from patients. These studies and others point to an increasing trend in modern biology in which high-throughput genome scale comprehensive biological information is incorporated into databases for constructing and reconstructing pathways and networks of interacting pathways that constitute a physiological

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response to environmental perturbation at the organism or systems levels. Drug Dev. Res. 62:124–133, 2004.

Key words: systems biology; genomic medicine; microarray; predictive; personalized

NEW ERA IN BIOMEDICAL RESEARCH

The massive efforts in partial DNA sequencing of expressed genes from cDNA libraries to generate expressed sequence tags (ESTs) [Adams et al., 1992], the completion of the human genome sequence [Lander et al., 2001; Venter et al., 2001], and the characterization and annotation of full-length human cDNAs [Imanishi et al., 2004] together have led to an explosive growth in sequence information, the identification of potentially all the genes and transcripts in the human genome, and their functional annotation. These efforts have already greatly facilitated biomedical research on the whole and will have significant impact on biomedical research in this new millennium. Information from post-genomic functional analysis of genes coupled with all the available biological information in the published literature are captured into unified databases so that biochemical pathways and networks of interacting pathways can either be constructed or reconstructed for in silico systems biology modeling [Hood, 2003; Weston and Hood, 2004]. As a result, it is envisioned that querying such databases would facilitate the identification of targets and pathways in response to perturbations in a system, thus yielding testable hypotheses for experimental validation and application in drug discovery and genomic medicine.

Despite the exponential growth in gene and protein information, knowledge about the pathways and the network of pathways that they function in are lagging behind. Recent advances in microarray technology have revolutionized genomic and proteomic research, yielding functional genomic datasets that are poised for genome-scale analysis [Brown and Botstein, 1999; Chee et al., 1996; Schena et al., 1998]. A major caveat amidst these optimisms is that, currently, there are still insufficient genomic datasets generated from microarray studies. Furthermore, data from most microarray studies lack robustness due to limited sample size and insufficient data points in the experimental designs. Hence, such results are not always informative and are difficult to decipher. These shortfalls are a direct result of the high cost incurred in conducting microarray studies, as well as a lack of expertise in guiding experimental design and array data analysis. It is hope that with time these deficiencies will be overcome so that large-scale biological data can be obtained from microarray analysis and then integrated with other biological information for in silico systems biology modeling.

LARGE-SCALE BIOLOGICAL DATA

Until recently, functional characterization of genes in biomedical research, using various conventional biochemical and molecular biological approaches, has been inherently slow [Walhout and Vidal, 1999]. Some recent innovative biotechnological advancements coupled with high-throughput adaptation and automation of these assays have facilitated the pace of biomedical discovery. For example, the use of a high-throughput yeast two-hybrid interaction screen has resulted in the comprehensive mapping of protein-protein interactions that reveals a network of proteins with similar functional pathways, thus enhancing our understanding of the physiological roles of genes and cellular function at the genome scale and organismal levels [Li et al., 2004; Uetz et al., 2000].

Further, the discovery of DNA microarray leverages an unprecedented view of the genome in response to perturbation [Chee et al., 1996; Iyer et al., 1999; Lockhart et al., 1996; Schena et al., 1995; Spellman et al., 1998]. In a single analysis, genome-wide alterations in gene expression can be identified using statistical algorithms [Eisen et al., 1998] that reveal distinct patterns of gene changes, which coalesced into functionally related clusters of genes associated with specific biochemical or genetic pathways [Iyer et al., 1999; Spellman et al., 1998]. These networks of genes may have implications about their dynamics and cellular function. Therefore, networks of regulatory genes may facilitate the description of function and genome functional annotation [Brazhnik et al., 2002; Diehn et al., 2003; Wyrick and Young 2002].

In addition to in vitro experimental and animal models, expression-profiling studies by DNA microarray are increasingly applied to disease models in human using clinical specimens from patients [Alizadeh et al., 2000; Beer et al., 2002; Chin et al., 2002; Golub et al., 1999; Konstantinov et al., 2004; Nomura et al., 2003; Rosenwald et al., 2002; van 't Veer et al., 2002; van de Vijver et al., 2002]. These expression profiles enabled molecular classification of diseases as well as prediction of disease and treatment outcomes

that otherwise could not be easily accomplished by traditional experimental biology and clinical practice. DNA microarray analysis with tissue specimens from patients potentially may also yield expression genomic results that are systems-wide and reflects cellular and physiological responses at the organismal levels. Hence, the results and information generated from these studies will be suitable for in silico modeling in systems biology. The combination of laboratory and clinical information will be a rich resource for data warehousing. Therefore, interrogating these databases for matching patterns of networks of regulatory genes and pathways, and signature patterns, may produce hits that engender hypotheses for further testing, validation, and subsequent application in molecular and drug target discovery, and in predictive and personalized medicine.

ON THE BENCH

Mutant Genetics and Expression Profiling

Effects of the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) in cells have been intensely investigated. Many genes have been shown to be induced by TPA but whether they are involved in TPA-mediated differentiation has not been systematically studied [Ventura and Maioli, 2001]. In previous gene expression profiling studies by DNA microarray, we examined the expression profiles elicited by TPA in the human leukemia HL-60 cells compared to its TPA-resistant mutant variant HL-525 cells [Zheng et al., 2002]. We found that TPA-induced temporal changes in gene expression during differentiation of HL-60 cells were hierarchical and pyramidal, using polynomial regression model analysis (Fig. 1) [Zheng et al., 2002]. Elevated expression of small cytokines and interleukins in HL-60 cells suggests inflammatory response and differentiation induction stimulated by TPA. However, these genes were not induced in the TPA-resistant mutant HL-525 cells, thus suggesting that they may be required for TPA-induced differentiation in the wild-type HL-60 cells. We further observed that some genes of the transforming growth factor-β (TGF-β)-signaling pathway were altered in their expression in HL-60 cells. Moreover, these genes were also induced and

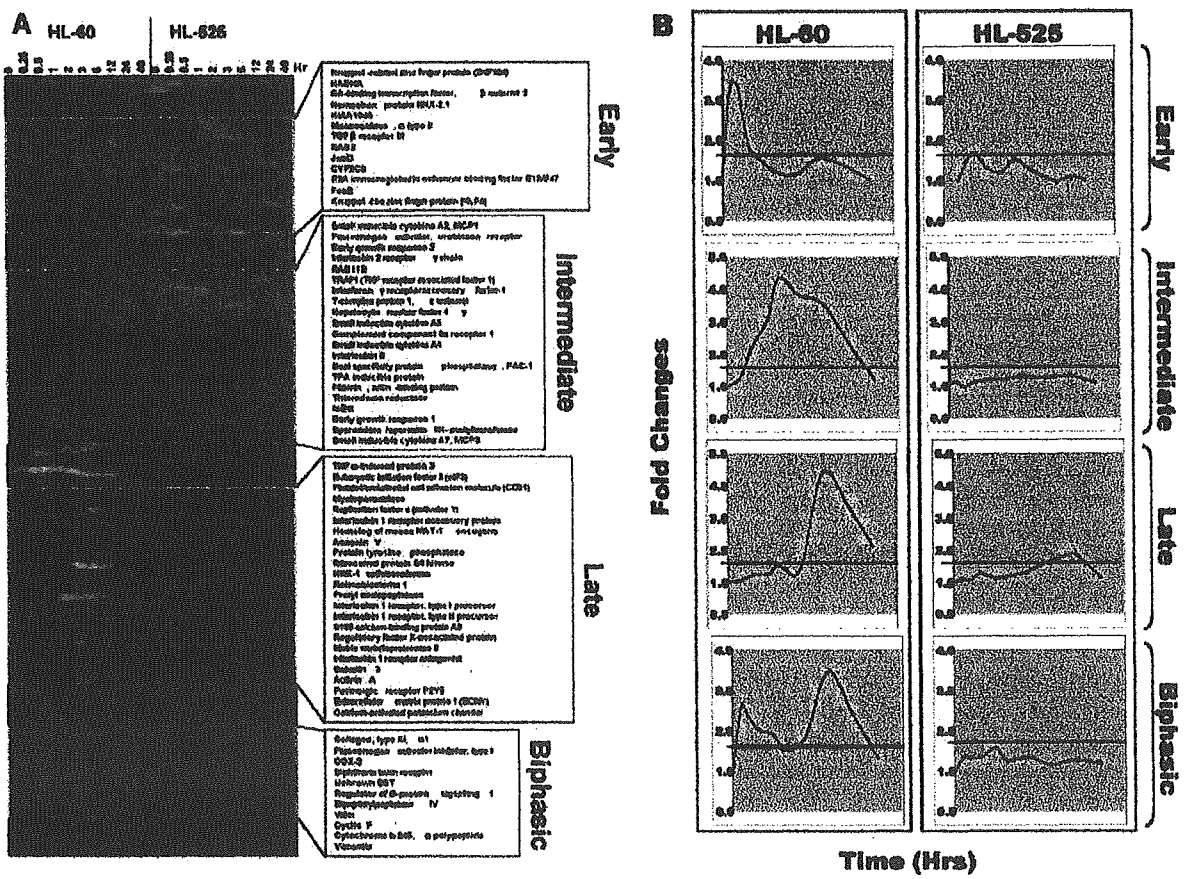

Fig. 1. Expression profiling of TPA-induced differentiation by DNA microarray. Gene expression profiles of TPA-induced HL-60 and its TPA-resistant mutant HL-525 cells. **A:** Dendrogram of time-course analysis of HL-60 and HL-525 cells in response to TPA from 15 min to 48 h. **B:** Polynomial regression modeling of microarray data showing early, intermediate, late, and biphasic (cell cycle) clusters of gene changes.

expressed in HL-525 cells, hence they may not be involved in TPA-mediated differentiation.

Even though the above expression-profiling studies were conducted using microarrays that only contained approximately 4,000 EST elements, nonetheless, the mutant genetic approach coupled with detailed kinetics analysis by microarray expression profiling revealed unambiguously a robust and informative network of regulatory genes that seemed to be important for TPA-induced differentiation in leukemia.

MECHANISMS OF GREEN TEA POLYPHENOL IN CANCER PREVENTION

The above example shows that transcription or gene expression profiling facilitated the identification of a previously unrecognized network of regulatory genes with a related function that mediates TPA-induced cellular differentiation. TPA-induced gene expression has been investigated intensely and many genes have been identified to be targets of TPA. Our experiment confirmed the expression of a large number of these genes and further distinguished them into distinct clusters that are functionally consistent with the mechanisms of TPA. We examine further whether expression profiling with less well-characterized small molecules might also yield similarly informative networks of genetic pathways that point to their mechanisms of action.

Some epidemiological studies suggested that regular green tea consumption might lower the incidence of cancer [Kohlmeier et al., 1997; Yang et al., 2002; Yang and Wang, 1993]. These results were further supported by studies in animal models that demonstrated the efficacies of green tea and its major polyphenolic constituent, *epi*-gallocatechin gallate (EGCG), in preventing the development of a number of different cancers [Ahn et al., 2003; Isemura et al., 2000; Paschka et al., 1998; Yang et al., 2002]. Various biochemical pathways have been shown to be affected by green tea and EGCG [Cao and Cao 1999; Chung et al., 1999, 2001; Islam et al., 2000; Liang et al., 1999; Lin et al., 1999; Naasani et al., 1998, 2003]; however, the precise mechanisms of EGCG in cancer prevention are still not fully understood. Moreover, preparation of EGCG in aqueous solution results in its oxidation and the production og hydrogen peroxide (H_2O_2) and other oxidative products, thus further confounding the search for its cellular mechanisms [Dashwood et al., 2002; Hong et al., 2002; Long et al., 2000; Yang et al., 1998a, 2000].

To distinguish the action of EGCG from that of H_2O_2, we treated the human bronchial epithelial BES21 cells with EGCG either in the presence or absence of catalase, which catalyzes the decomposition of H_2O_2, in a time-course kinetics analysis [Vittal et al., 2004]. We found that many genes including those of the TGF-β-signaling pathway, were H_2O_2-dependent, because the effects were abolished by catalase, whereas expression of some genes distinctly associated with the bone morphogenetic protein (BMP)-signaling pathway were not affected by catalase treatment (Fig. 2). Temporal changes in gene expression by EGCG were also hierarchical and pyramidal. We validated some of these changes in gene expression from DNA microarray analysis by showing that EGCG and H_2O_2 differentially transactivated the BMP- and the TGF-β-response element promoter reporters, respectively. We also confirmed recently the transactivation of the FK506 binding protein 5 gene promoter, a gene of the BMP-signaling pathway, by EGCG (unpublished results).

Many mechanisms have been proposed for EGCG, but it is unclear which of these are H_2O_2-dependent and which are H_2O_2-independent. Our results reveal distinctly that a network of regulatory genes of the BMP-signaling pathway may be induced specifically by EGCG in an H_2O_2-independent manner, while activation of gene expression of the TGF-β pathway may be attributed to H_2O_2, an oxidative product of EGCG (Fig. 3). Therefore, the elucidation of gene expression changes between the H_2O_2-dependent and H_2O_2-independent responses not only helps us better understand the cancer chemopreventive action of EGCG, but also facilitates the identification of a cluster of novel targets for EGCG.

GENOMIC MEDICINE: ERA OF PREDICTIVE AND PERSONALIZED MEDICINE

The examples of TPA and EGCG demonstrated that expression-profiling studies in the laboratory facilitates the elucidation of the mechanisms of action for small molecules as well as the identification of novel molecular and drug targets. Cumulative accrual of this information for databases will make possible the implementation of in silico systems biology modeling for querying the mechanisms and targets of novel and anonymous small molecules by pattern matching, much as is done for gene or protein sequence searches today. It is conceivable that mechanistic as well as molecular and drug target information derived from expression genomics analysis may contain additional knowledge or signature expression patterns for drug-induced metabolism that could influence treatment response and outcome. However, whether expression of genomic data derived from in vitro cell culture or animal models may be applicable or extrapolated to humans remains to be determined.

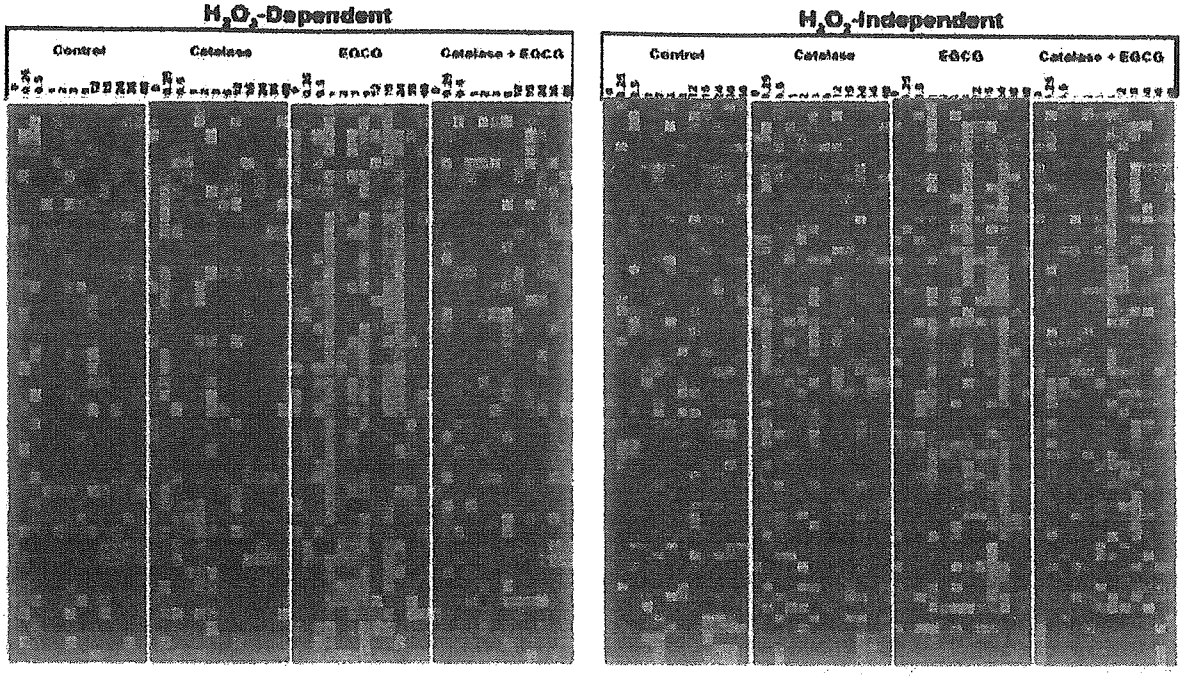

Fig. 2. Mechanisms of EGCG in cancer prevention by DNA microarray. Transcription profiles of the transformed human lung bronchial epithelial 21BES cells in response to EGCG in the absence or presence of catalase. Treatment with catalase abolished the induction of a number of genes that are probably due to H_2O_2 produced by oxidation of EGCG in culture system (H_2O_2 -dependent). Genes whose expression are not affected by catalase (H_2O_2 -independent) are most likely induced by EGCG.

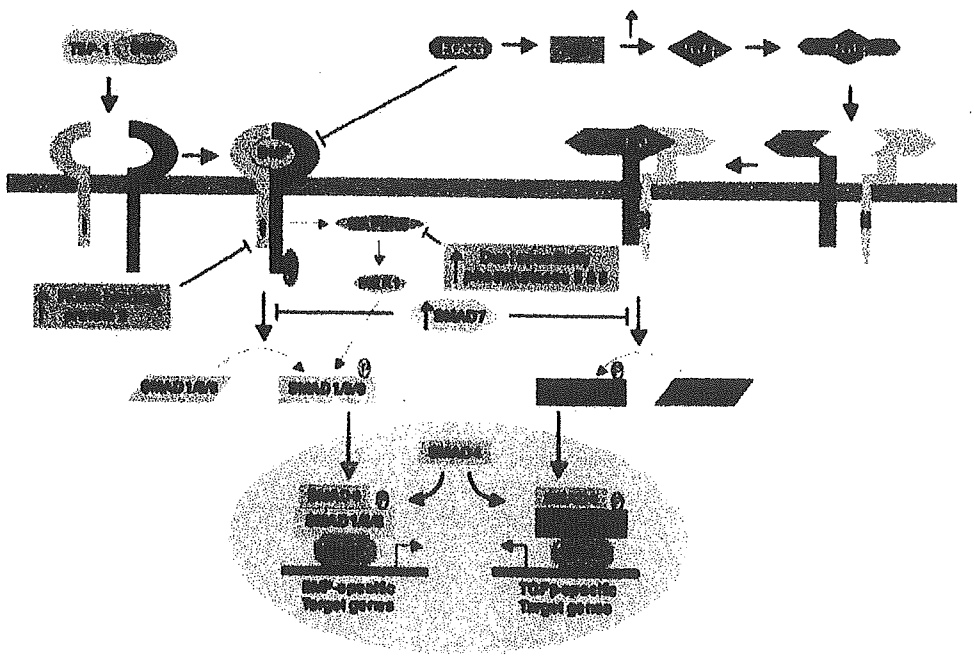

Fig. 3. Identification of pathways affected by EGCG. Expression profiling of cells treated with EGCG in a time course analysis as in Figure 2 led to the identification of H_2O_2 -dependent and H_2O_2 -independent genes that specifically affect either the BMP- or the TGF-β-signaling pathways, respectively.

Ideally, kinetics analysis derived from human samples will be most informative regarding treatment response and propensity for toxic side effects. However, it is difficult to conduct such studies with human subjects due to various difficulties including ethical issues and tissue accessibility. Nevertheless, expression profiling is increasingly applied in human clinical specimens, largely in cancer. Therefore, expression genomic data from human tissue specimens in combination with in vitro laboratory data will be a robust resource for in silico systems biology modeling to query and predict response to treatment, outcome, and susceptibility to toxicity. It is envisioned that databases containing expression genomic data coupled with other genomic information such as single nucleotide polymorphisms (SNPs), genomic sequence, and proteomic profile will be the cornerstone of genomic medicine, which would enable a physician to prescribe the right drug, at the right dose, for the right patient, in the near future.

PREDICTIVE MEDICINE

Predicting Response to Radiotherapy

Here, we illustrate the use of expression genomics coupled with a pattern recognition algorithm in the analysis of human cancer specimens for predicting treatment response. We asked whether a voice/speech pattern recognition algorithm used in the telecommunication industry could be adapted for expression genomic data analysis for molecular classification and prediction of response to radiotherapy in patients with cervical cancer [Wong et al., 2003].

A statistical approach to pattern recognition was applied in our studies, using a combination of linear discriminant analysis for training set, feature extraction by Bayesian parameter estimation, decision by nearest neighbor classification, and classifier performance evaluation. Patients were selected from each category, "sensitive" and "resistant," for training and feature selection. Testing was then conducted with the samples set aside from each category that were not used for training.

We obtained expression profiles from patients' primary cervical cancer samples, taken at the time of diagnosis and before radiotherapy, in a retrospective analysis. For classifying the tumor samples, we used gene expression data from cDNA microarrays containing 10,692 elements. To first test whether a voice/speech pattern recognition algorithm was suitable for microarray data analysis, we compared the expression profiles of normal cervices derived from hysterectomy with cervical cancer samples. Pattern recognition algorithm correctly classified all the samples by their expression profiles as either normal or cancer (Fig. 4).

Cervical cancer is clinically staged according to the FIGO system into four stages. We then showed by pattern recognition that some of the samples could also be correctly classified into Stage IB or Stage IIB disease.

Next, patients who were given radiotherapy as primary treatment were analyzed for radiotherapeutic sensitivity (responder) or resistance (non-responder). Patients who did not respond to radiotherapy had a mean survival time of 22.2 months while the group sensitive to radiotherapy had a mean survival time of 66.5 months. Our results showed that pattern recognition correctly classified the samples to be either radiotherapy-sensitive or radiotherapy-resistant based on their gene expression profiles (Fig. 4). We identified a signature expression profile that can be predictive of response to radiotherapy in these patients. These signature predictors include a large number of genes with diverse physiological functions, which include transcription factors, and proteins with cytoskeletal, membrane, and cell structural functions. Further analysis of a larger group of cervical cancer patients treated by radiotherapy will be required to confirm our finding and to determine the direct role of these genes in resistance to radiotherapy.

Predicting Response to Chemotherapy

To further test the potential clinical application of pattern recognition and expression genomics in modeling and predicting treatment response, we examined here the expression profiles of primary ovarian cancers stratified into two groups based on their chemotherapeutic response [Selvanayagam et al., 2004].

The ability of cancer cells to develop intrinsic or acquired resistance to virtually every drug in cancer chemotherapy is a major problem that leads to treatment failure. It is clear that multidrug resistance in cancer is not attributable to the overexpression of the ATP-binding cassette (ABC)-superfamily of transporters alone [Gottesman et al., 2002], but may be associated with genetic alterations during tumor progression that is accompanied by changes in the expressions of a large number of genes [Kudoh et al., 2000]. Some of these gene changes may contribute to drug resistance by virtue of their extended normal cellular functions in transport [Sharma et al., 2003], metabolism [Doherty and Michael, 2003], signaling [Liem et al., 2002], and death and survival [Schmitt and Lowe, 2002; Tsuruo et al., 2003]. In ovarian cancer, emergence of drug resistance results in death for more than 90% of patients with metastatic disease. The poor prognosis has prompted major efforts to identify

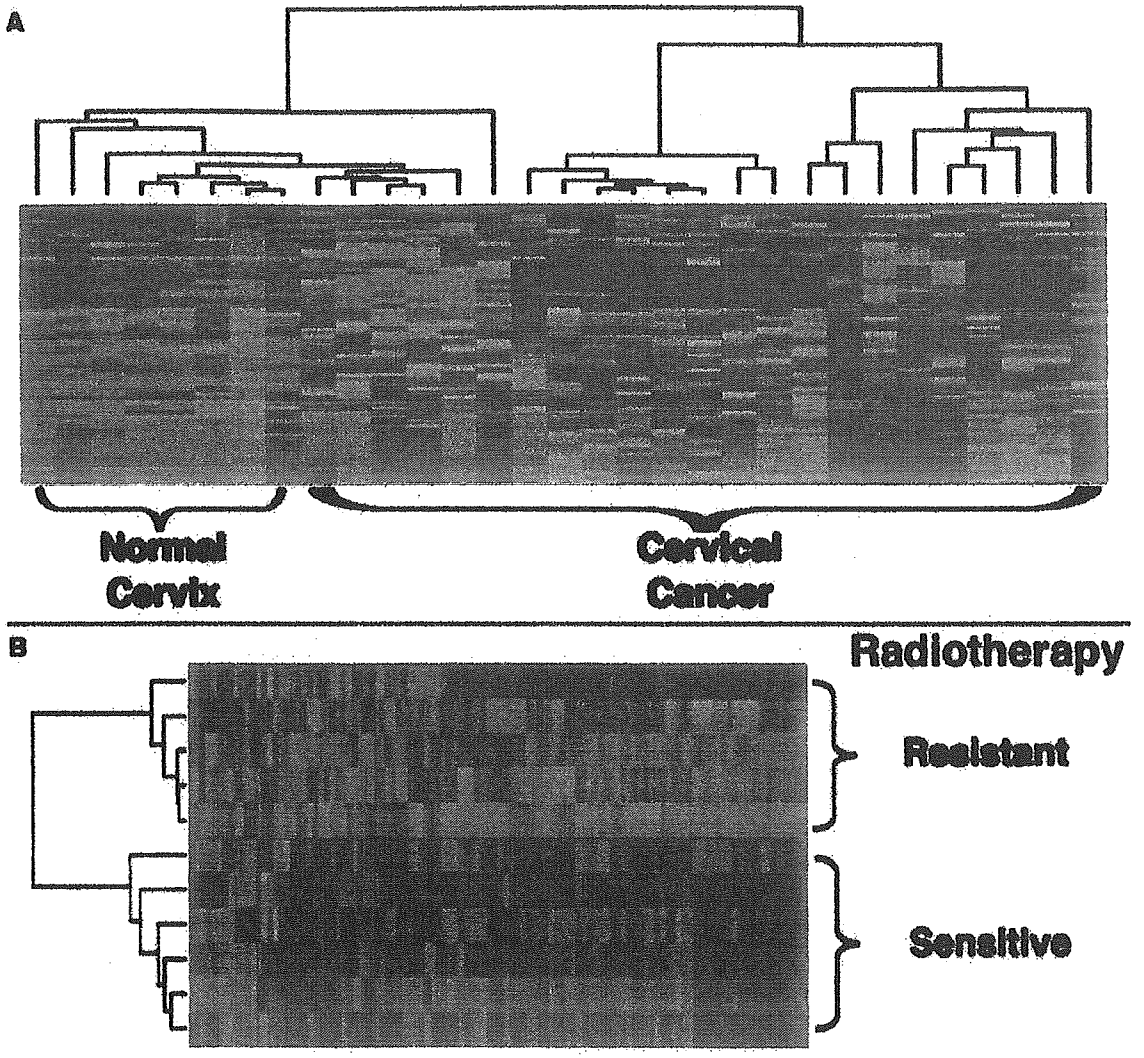

Fig. 4. Expression genomics of cervical cancer. **A:** Molecular classification of cervical cancer by DNA microarray. Normal cervix and cervical cancer samples were correctly classified by voice/speech/pattern recognition. **B:** Prediction of radiotherapy response in cervical cancer based on transcription profiles of primary cervical cancer samples obtained at time of diagnosis before therapy was given.

prognostic factors, improve surgical staging, and develop adjuvant therapies that could improve patient outcome [Ozols, 2000].

In this retrospective analysis, patients were given either Cisplatin/cyclophosphamide, or Carboplatin/taxol, or Cisplatin/Taxol. Pattern recognition analysis of the expression profiles correctly predicted the response of the two groups of patients as either chemotherapy sensitive or chemotherapy resistant (Fig. 5).

It is noteworthy that some predictor genes of the signature expression pattern of drug-resistant cancers, which include transcription factors that bind DNA, identified from patients who did not respond to treatment are functionally involved in the regulation of gene expression. This signature expression pattern is reminiscent of the expression profiles found in ovarian cancer cell lines exposed to Cisplatin (unpublished data), thus suggesting that these nucleic acid–binding proteins may have an important role in conferring either intrinsic or acquired drug resistance in ovarian cancer. We also observed an increase in glutathione S-transferase expression, which is known to confer resistance to Cisplatin.

Whether the transcriptional changes in the expression of these predictor genes are associated with the etiological causes of drug resistance or treatment failure remains to be determined in future studies with a larger cohort of ovarian cancer patients. Nevertheless, these proof-of-principle pilot studies provided evidence of the potential application of expression

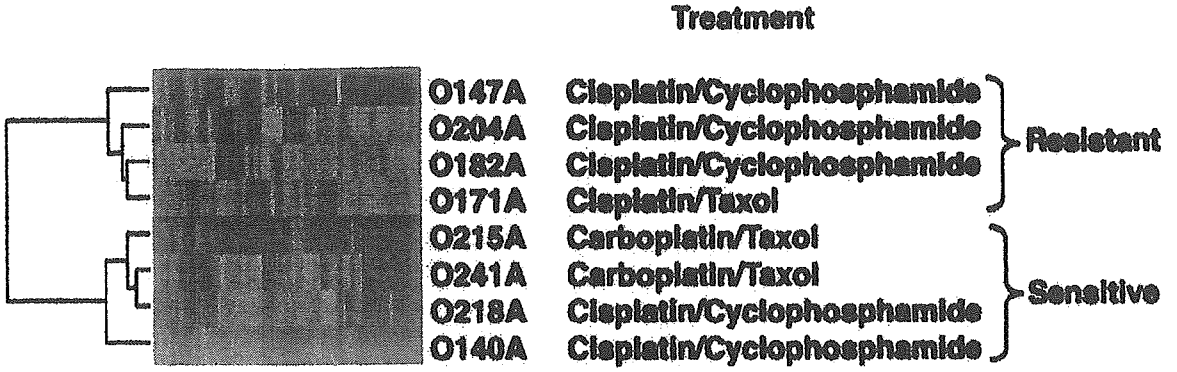

Fig. 5. Prediction of chemotherapeutic response in ovarian cancer. Pattern matching algorithm correctly predicted treatment response and the displayed dendrogram showed patients who either responded to or failed the indicated treatment regimens.

genomics diagnostically in the molecular classification of diseases and prediction of treatment response.

PREDICTING AND PERSONALIZING TREATMENT

Emergence of resistance in cancer treatment is a major unresolved problem. Failure to respond to treatment whether by radio- or chemotherapy is often the result of the empirical nature of cancer therapy or the inability to foresee or predict response of individuals' cancer. Therefore, monitoring the expression profiles of cancer and identifying signature patterns that are the culprits of treatment resistance will not only provide insights into the mechanisms of resistance, but also ultimately help guide and improve cancer treatment.

The current one-size-fits-all approach in the clinical treatment of cancer and other diseases does not take into account whether patients may or may not respond to the treatment modality. We reviewed here the application of expression genomics derived from an individual patient's unique gene expression information, which promises to offer a much-needed new avenue in the clinical treatment of diseases including cancer, thus circumventing the unpredictable trial-and-error and one-size-fits-all approaches of clinical medicine today. Hence, patients will be spared from unnecessary treatment and exposure to the associated toxic side effects of drug treatment.

Despite these advances, there are currently no clinical markers that enable physicians to accurately predict, a priori, whether patients would respond to treatment. Undoubtedly, monitoring gene expression profiles by DNA microarray in human cancer as well as other diseases will be the gateway genomics approach to diagnosis, which can provide pharmacogenomics information for accurately predicting treatment outcome, thereby bringing an end to the practice of the trial-and-error and one-size-fits-all approaches in the treatment of diseases. Patients whose expression profiles exhibit a pattern associated with resistance to treatment will be given an alternative or supplementary modality of treatment that may result in improved responsiveness or cure, thus personalizing treatment for individuals based on their gene expression patterns.

CONCLUSION

We have reviewed here the application of expression genomics in elucidating the mechanisms of action of small molecules and identification of novel molecular targets. We also reviewed two proof-of-principle studies demonstrating the prediction of treatment outcomes by monitoring the gene expression profiles of cancer. We further discussed the integration of biological information or systems-wide data, and the expression of genomic data of clinical specimens for in silico modeling of physiological response to small molecule perturbations, which may provide insight into the networks of regulatory genes, pathways, and interacting pathways that point to their mechanisms of action, and may help predict either treatment response or susceptibility to toxicity.

It is increasingly clear that the development and discovery of new drugs for the treatment of diseases alone is strategically inadequate, for there are diverse genetic differences between individuals that may affect treatment response and susceptibility to toxicity. Hence, it is foreseeable in the future that disease treatment will be closely monitored and coordinated with patients' genomics information so that treatment response and outcome and adverse effects can be predicted, and, subsequently, treatment can be personalized to achieve the maximal beneficial effects or cure for the patients.

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Amplicon profiling reveals cytoplasmic overexpression of MUC1 protein as an indicator of resistance to platinum-based chemotherapy in patients with ovarian cancer

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Abstract. Chromosomal gains of 1q21-q22 and 13q12-q14 were closely related with the chemoresistance of patients with ovarian cancer in our previous CGH (comparative genomic hybridization) study. We conducted the present study to determine the amplified genes on chromosome 1q. Comparisons of relative copy numbers of clinically platinum-sensitive ovarian tumors (CR-group, n=14) and platinum-resistant tumors (PD-group, n=14) were carried out using real-time PCR from ten different gene loci on chromosome 1q. Increased copy numbers were frequently observed in PD-group tumors, especially in the region between WI-8123 and *MUC1*. Relative copy number of MUC1 over 1.5 was observed in 13 (92%) of 14 PD-group tumors and 3 (21%) of 14 CR-group tumors ($p<0.05$). Moreover, cytoplasmic expressions of MUC1 protein were significantly higher in PD-group than those in CR-group ($p<0.01$). We concluded that the cytoplasmic overexpression of MUC1 might be an indicator of resistance to platinum-based chemotherapy and a prognostic marker in ovarian cancer.

Introduction

Ovarian cancer is the third most common malignancy of the female genital tract, but survival rates of affected patients are the lowest in the patients with genital tract carcinomas. It is the fifth most common cause of death among women (1). The mechanisms of chemoresistance is so complicated that the clinical response to chemotherapy is not predictable. *In vitro* studies suggested that acquired resistance to cisplatin is associated with increased levels of glutathione and glutathione-S-transferase activity, increased metallothionein and decreased accumulation of cisplatin (2).

In our previous study, gains of 1q21-q22 and 13q12-q14 in PD-group tumors were observed in significantly high abundance, compared with those in CR-group tumors (3). Cisplatin-resistance related genes seemed to be located on chromosomal regions of 1q21-q22 and 13q12-q14. Many candidate genes which might be involved in chemoresistance are located on the chromosomal region of 1q21-q22, including *BCL-2*-related myeloid leukemia sequence (*MCL-1*), the polymorphic mucinous tumor-associated gene (*MUC-1*), and the papillary renal cell carcinoma gene (*PRCC*).

We conducted the present study to determine whether highly amplified genes exist on 1q21-q22 by real-time PCR with the primers located on this region. Moreover, MUC1 the highly amplified gene located on this region, was immunohistochemically examined to see if there is a relationship with cisplatin-resistance in ovarian cancer patients.

Material and methods

Patients, chemotherapy, and response evaluation. Of 71 patients with primary epithelial ovarian cancer treated at the National Defense Medical College Hospital (Saitama, Japan) between 1993 and 1997, the following patients were selected: a) patients who received no prior chemotherapy prior to the any surgical therapy; b) patients who harbored any residual tumors after initial debulking surgery; and c) patients treated with six courses of cisplatin-containing chemotherapy (described below) after the initial surgery. The patients were then grouped into the following four categories according to their response to chemotherapy: a) CR, complete response; b) partial response; c) stable disease; and d) PD, progressive disease (4). Only patients in the CR (n=14) and PD (n=14) were included in the study. The chemotherapy regimen for the patients was as follows: one cycle consisted of a drip infusion of 50 mg/m^2 cisplatin for 3 h accompanied by an i.v.

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Abbreviations: CGH, comparative genomic hybridization; CR, complete response; PD, progressive disease

Key words: real-time PCR, comparative genomic hybridization, chemoresistance, ovarian cancer, MUC1 protein

Table I. Patient characteristics.

	CR-group	PD-group	p-value
Age			
median	50.3	51.2	
range	20-69	42-74	0.381[b]
Stage			
II	3	1	
III	10	10	
IV	1	3	0.368[c]
Histology[a]			
S,E	9	6	
C,M	5	8	0.256[c]
Grade			
1	2	1	
2	6	5	
3	6	8	0.157[c]
Residual tumor			
<2 cm	11	7	
>2 cm	3	7	0.298[c]
lq21-q22 gain			
yes	2	9	
no	12	5	0.0068[c]

[a]S, serous cystadenocarcinoma; E, endometrioid adenocarcinoma; C, clear cell adenocarcinoma; M, mucinous cystadenocarcinoma. [b]Exact score test. [c]Chi-squared test or Fisher's exact method.

Table II. Primer sequences used in real-time PCR.

Name of primers	Sequence
D1S442	5'-AACAAAGCTGGACTGGTAATC (sense) 5'-CAGTGTCACACAACTGGTTG (antisense)
WI-8123	5'-TTCTCTGGGAAAACCCCTG (sense) 5'-ATTGCCCATCATAGACTTTTTACA (antisense)
MUC1 (exon2)	5'-TACTCCTACCACCCTTGCCA (sense) 5'-GAGAAGTGCTGTGATTGGAGG (antisense)
H59801	5'-TAGGCCCGGCTGTGGCT (sense) 5'-AGCCCCTCACAGGCATCACT (antisense)
NTRK1	5'-TAGGCCCGGCTGTGCTGGCT (sense) 5'-AGCCCCTCACAGGCATCACT (antisense)
WI-8997	5'-GTGGCCATCGATCTGGAC (sense) 5'-ACCATGAGACACACAGTTCTGG (antisense)
WI-9108	5'-CTCTTCCCCCTGACTCCC (sense) 5'GCAGAAAGAGAAACAATTTAAATGG (antisense)
WI-9232	5'-GAATTGATGCCCTTCGATGT (sense) 5'-GTATCAATTTTCCTCGACTGTGC (antisense)
WI-9272	5'-CAAAGATCTGCTCCTCGCTC (sense) 5'-AGTGGTGGCTCCCACCTAG (antisense)
WI-9317	5'-GCTGTTAGTGAGATGGTGAAGC (sense) 5'-AAAAAGTTCAAGAGTCAGCAGTAGA (antisense)

injection of 50 mg/m^2 doxorubicin and 500 mg/m^2 cyclophosphamide and 6 cycles were given every 4 weeks (4). CR-group consisted of six serous, two mucinous, four endometrioid, and two clear cell adenocarcinomas. PD-group contained five serous, two mucinous, one endometrioid, and six clear cell adenocarcinomas. The patient characteristics are shown in Table I. In the previous CGH analysis, the numbers of patients harboring chromosomal gains of 1q21-q22 were 2 (14%) of 14 patients with complete response (CR) and 9 (64%) of 14 patients with progressive disease (PD), respectively (3).

DNA extraction. High molecular weight genomic DNAs were isolated from primary tumors of the first debulking operation using phenol-chloroform methods (5). All samples were examined by 1% agarose gel electrophoresis for conservation of high molecular weight.

Real-time PCR. The real-time PCR was used to validate differential expression of genes located on chromosomal region of 1q21-q22. All samples of ovarian cancer patients showed chromosomal gains of 1q21-q22 in our previous study with CGH. Analysis was carried out using the ABI Prism 7700 Sequencer Detector System according to the manufacturer's recommended protocol (ABI/Perkin Elmer, Foster City, CA, USA). Each reaction was run in duplicate. For the need to measure precisely the concentration of the sample DNA, ß2-microglobulin (chromosome 15q21-22.2) was used as an internal control gene. Chromosome 15 is rarely involved in the karyotyping of ovarian cancers (6-8). The comparative threshold cycle (C_T) method was used for the calculation of amplification fold as specified by the manufacturer. The standard curves used to measure copy number in this study were constructed from DNA of normal female placental tissue. DNAs of placental tissue were diluted in five concentrations; 50, 25, 12.5, 6.25, and 3.13 ng/µl. To avoid the increased and decreased validity, test samples were