UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Instituto de Ciências Básica da Saúde

Comissão de Graduação de Biomedicina

ASSINATURA GÊNICA DE RESISTÊNCIA À CISPLATINA ENVOLVE A REDE DA COFILINA-1 EM CÂNCER DE PULMÃO DE NÃO-PEQUENAS CÉLULAS

MARCO ANTÔNIO DE BASTIANI

Trabalho de Conclusão do Curso em Biomedicina

Orientador: Dr. Fábio Klamt

PORTO ALEGRE

2012

Agradecimentos

Agradeço a minha mãe e minhas irmãs pelo apoio e carinho durante esses anos de graduação; por sempre me motivarem em minhas empreitadas e confiarem em mim, quaisquer que sejam minhas decisões; por estarem presentes, apesar da distância e das dificuldades. Também agradeço a meu cunhado Julio Niderauer pela amizade e por ser uma pessoa em quem posso confiar e contar.

Agradeço ao pessoal do grupo do professor Fábio – Carolina Muller, Liana Marengo, Ricardo Rocha, Valeska Aguiar e demais – pela amizade e ajuda durante os quatro anos que estou no laboratório e com certeza após a graduação. Em especial, preciso agradecer ao colega Matheus Becker por toda a ajuda e ensinamentos desde que entrou no lab. até o TCC, e certamente por depois da graduação também. Ainda, agradeço as colegas que recentemente deixaram o grupo para seguir outros caminhos – Fernanda Lopes e Giovana Londero – pelas boas lembranças e amizade formada. Também agradeço ao pessoal do laboratório 32 pelas boas lembranças e ao restante do pessoal do laboratório 24 pela disposição e ajuda no dia a dia de trabalho.

Agradeço ao professor Fábio Klamt por ter me acolhido em seu grupo de pesquisa; por confiar e investir em meus potenciais e permitir que os desenvolva; por ser alguém em que posso aspirar como profissional e como pessoa; e pela amizade e apoio.

Agradeço a todos que de alguma forma contribuíram para minha formação profissional e pessoal nesse tempo único da vida que é a graduação.

Índice

Resumo

O câncer de pulmão é a neoplasia maligna mais insidiosa da oncologia, sendo responsável pelo maior número de mortes relacionadas a câncer no mundo. Este câncer pode ser dividido em dois tipos: câncer de pulmão de células pequenas e câncer de pulmão de não-pequenas células (CPNPC). A maioria dos tratamentos de primeira linha atuais baseia-se em agentes alquilantes derivados de platina, como a cisplatina e a carboplatina, utilizados sozinhos ou em conjunto com outras drogas antineoplásicas. A ação antitumoral dos derivados de platina ocorre basicamente em nível de DNA, provocando a formação ligações cruzadas principalmente intra-fitas. Independentemente do regime utilizado, entretanto, é inevitável o surgimento de resistência e, por ser o padrão ouro da terapêutica, a resistência a derivados de platina é um fator importante no desenrolar do tratamento. Três são os principais mecanismos descritos de resistência a esses compostos: aumento do efluxo, da inativação intracelular da droga e aumento da taxa de reparo do DNA. Além disso, outro mecanismo proposto é o aumento da expressão de carreadores da membrana nuclear, que auxiliariam na entrada de proteínas de reparo. Trabalhos anteriores de nosso grupo identificaram a proteína cofilina-1 como um possível biomarcador candidato para CPNPC e esses trabalhos também demonstraram uma possível relação entre os níveis de expressão da proteína cofilina-1 e resistência a agentes alquilantes, onde células que possuem alta expressão (e imunoconteúdo) da proteína possuem uma maior resistência a esses agentes quimioterápicos. Por esse motivo, o objetivo deste trabalho é avaliar a rede de interação gênica da cofilina-1 com relação aos principais mecanismos de resistência descritos pela literatura em um modelo *in silico* utilizando dados de expressão gênica de microarranjos. Para isso, dados de microarranjo de modelos celulares e biópsias foram extraídos do repositório público *Gene Expression Omnibus* (GEO) e analisados no software ViaComplex. Esse programa utiliza-se de redes de interação geradas na ferramenta *Search Tool for the Retrieval of Interacting* Genes*/Proteins* (STRING) e do dado obtido a partir do microarranjo para avaliar o padrão de expressão de grupos de genes. Além do ViaComplex, também realizamos análises de correlação para investigar a relação de nossos genes de interesse com o processo de resistência à droga. Nossos resultados mostram que o grupo de genes relacionados à cofilina-1 comporta-se similarmente ao grupo de genes relacionados ao reparo por NER na maioria das análises realizadas. Ainda, nossas análises sugerem que o padrão de expressão dos genes das redes varia

diferentemente dependendo do agente alquilante e do tipo histológico do tumor. Finalmente, análises dos bancos de dados de biópsias reforçaram dados da literatura que afirmam uma relação entre cofilina-1 e desfecho desfavorável para pacientes em estágios iniciais de CPNPC.

I. Introdução

1. Câncer de Pulmão

No século XIX, o câncer de pulmão era uma doença rara, representando somente 1% de todos os cânceres vistos em autópsias. Entretanto, já no início do século passado, a incidência de tumores malignos de pulmão aumentou tanto entre homens quanto entre mulheres. Embora a associação entre o cigarro e o câncer de pulmão era suspeitada pelos médicos na década de 1930, a causa do aumento dramático não fora bem estabelecida até estudos epidemiológicos na década de 1950 mostrarem uma forte associação entre o fumo e o câncer de pulmão (Sun et al., 2007). Atualmente, o câncer de pulmão é uma doença insidiosa altamente letal, com o percentual de pacientes que apresentam sobrevida superior a cinco anos variando entre 13 a 21% em países desenvolvidos e entre 7 a 10% em países em desenvolvimento. O principal fator de risco para o seu desenvolvimento é indubitavelmente o tabagismo. Estudos mostram que tabagistas apresentam cerca de 3 a 5 vezes mais risco de desenvolver carcinoma de pulmão quando comparados com pessoas que nunca fumaram, sendo que esse risco diminui significativamente em ex-tabagistas e é proporcional ao quão cedo na vida o indivíduo cessou o hábito (Khan et al., 2010).

O tratamento para o CPNPC é determinado pelo estadiamento da doença e classificados de acordo com a descrição TNM dentro de categorias e estágios, sendo que o tipo de estágio da doença envolve uma combinação de fatores que levam em consideração características patológicas do tumor (Mountain, 1997). Por determinar o curso da terapia, esse procedimento é de fundamental importância clínica e está sujeito a revisões e alterações periódicas a fim de aumentar os benefícios para o paciente (Nair et al., 2011). A rescisão cirúrgica continua sendo a principal forma de tratamento para os estágios iniciais e doenças localizadas, contudo a quimioterapia sistêmica também beneficia esses pacientes (Arriagada et al., 2004). Da mesma forma, terapias sistêmicas multimodais tornaram-se uma prática comum para doenças regionalmente avançadas. Embora pacientes com doença avançada e metastática sejam somente candidatos a tratamentos quimioterápicos paliativos, estes apresentam evidências de melhoras na sobrevivência e qualidade de vida dos pacientes (Ramalingam and Belani, 2008).

Os benefícios da quimioterapia baseada em agentes alquilantes (com base em platina) como um tratamento de primeira escolha para pacientes com CPNPC avançado foi reportado pela primeira vez no final da década de 80. Mais evidências mostrando a eficácia dessas terapias foram demonstradas subsequentemente por meta-análise de vários ensaios clínicos randomizados. Esta análise demonstrou que a quimioterapia baseada em cisplatina foi associada com uma taxa de sobrevivência de 1 ano (Ramalingam and Belani, 2008). Por esse motivo, agentes alquilantes começaram a ser combinados com outros medicamentos no intuito de melhorar a sobrevida e a qualidade de vida dos pacientes.

2. Mecanismo de Ação da Cisplatina

Cisplatina é um complexo inorgânico neutro e planar que reage com o DNA para induzir efeitos biológicos característicos, os quais podem ser de ativação dos mecanismos de reparo do DNA, com consequente sobrevivência celular, ou de ativação irreversível das cascatas apoptóticas. Contudo, a interação com o DNA só ocorrerá após uma série de reações espontâneas da molécula de cisplatina com o meio, que envolvem trocas sequenciais dos ligantes *cis*-cloro com moléculas de água (Kartalou and Essigmann, 2001; Rabik and Dolan, 2007). A forma resultante é reconhecida como uma espécie altamente apta a reagir com seu alvo de interesse: o DNA. Entretanto, o estado reativo da molécula também pode interagir com muitos nucleófilos endógenos, como glutationa (GSH), metionina, metalotioneína e outras proteínas.

A ação citotóxica da cisplatina é descrita pela sua interação com sítios nucleofílicos N7 das purinas no DNA, formando interações proteína-DNA, ligações cruzadas inter- e intra-fitas e monoadutos com o DNA (Rabik and Dolan, 2007), os quais são as principais lesões responsáveis pela morte celular (Siddik, 2003). Mais de 90% dos adutos ciplatina-DNA resultam em ligações cruzadas 1,2 d(GpG) intra-fitas, as quais modificam a estrutura tridimensional da molécula DNA e tornam o local um sítio de reconhecimento para várias proteínas. Estas proteínas de reconhecimento de dano incluem componentes do complexo de reparo MMR (sigla em inglês para *mismatch repair*) grupo 1 e 2 de proteínas não-histonas de alta mobilidade (HMG1 e HMG2), proteínas relacionadas ao reparo por excisão de nucleotídeos, dentre outras (Rabik and Dolan, 2007; Wang and Lippard, 2005).

3. Mecanismos de Resistência à Cisplatina

Como já mencionado, cisplatina é o padrão ouro da terapêutica em CPNPC e por esse motivo a resistência a essa droga significa um grande obstáculo para um regime terapêutico eficaz. A quimioresistência pode ser inata ou adquirida e pode referir-se a um único agente ou a uma classe de agentes que compartilhem mecanismos de ação antineoplásicos iguais ou similares. Uma parcela considerável dos pacientes com CPNPC eventualmente desenvolvem resistência contra os quimioterápicos aos quais eles estão expostos, mesmo que tenham apresentado uma resposta inicial boa. Por essa razão, é comum que esses pacientes recebam duas ou três linhas de terapia durante decorrer do tratamento (Chang, 2011). Existem três principais mecanismos de resistência descritos para a cisplatina: redução do acúmulo intracelular da droga, aumento da inativação intracelular da droga e aumento da eficiência do reparo ao DNA. Porém a literatura sugere que alguns outros sistemas também possam estar contribuindo nesse processo. Independentemente do processo, os mecanismos de resistência surgem como consequência de mudanças celulares que podem tanto impedir a droga de interagir com o DNA como interferir com a ativação da resposta aos efeitos citotóxicos, ou ainda ambos os processos.

3.1 Redução do Acúmulo Intracelular da Droga

Um primeiro nível de desenvolvimento de resistência é a seleção de mecanismos capazes de impedir ou diminuir o acúmulo intracelular do fármaco. Reduções na ordem de 20-70% foram documentadas em diversas linhagens celulares, e isso mostrou um aumento de resistência à cisplatina em um fator de 3-40 vezes. Entretanto, dada a natureza multifatorial da quimioresistência, a redução na acumulação da droga não parece ser necessariamente diretamente proporcional ao nível de resistência. De fato, o perfil insensibilidade à droga de um dado tipo de linhagem tumoral pode não incluir defeitos na acumulação da droga, ou esse sistema não ser o contribuinte majoritário da resistência. Por outro lado, em alguns cânceres, a redução na acumulação da cisplatina é o principal mecanismo, contabilizando 70-90% da totalidade da resistência (Siddik, 2003).

A causa da redução na acumulação intracelular da cisplatina nas células resistentes pode ser atribuída por uma inibição na captação da droga, por um aumento no efluxo da droga, ou por ambos. Um defeito no processo de captação, o qual é controlado por transportadores de cobre, parece ser comum e na literatura existe um crescente número de evidências apoiando o papel desses transportadores na resistência tumoral (Howell et al., 2010). O desenvolvimento de resistência como resultado de um aumento no efluxo da cisplatina é outro mecanismo possível de diminuição do acúmulo intracelular da droga. Nesse contexto, estão incluídas várias proteínas transmembrana, em especial proteínas associadas à resistência a múltiplas drogas (MDR, MRP e BCRP) da família de transportadores ABC (do inglês *ATP binding cassete*). No CPNPC, o grupo de proteínas MRP, composto de pelo menos 7 membros (MRP1-7), foram os principais alvos de investigação. Contudo, somente a MRP2 (cMOAT) parece estar envolvida na resistência à cisplatina, baseado observações de que células resistentes têm níveis elevados deste transportador. Ainda, um aumento de 10X na resistência foi demonstrado em células que super-expressam MRP2 e, além disso, a transfecção de RNA anti-senso contra a MRP2 aumenta a sensibilidade das células à cisplatina. Estudos envolvendo MDR/glicoproteína-p demonstraram-se inconclusivos quanto à resistência à cisplatina, apesar de a superexpressão desse transportador ter sido associada com uma resposta ruim à quimioterapia com essa droga (Siddik, 2003). Observa-se, portanto, que o mecanismo de redução do acúmulo intracelular da droga está fortemente associado com a resistência de diversas linhagens tumorais, não somente contra cisplatina, mas também contra diversos outros fármacos utilizados na clínica.

3.2 Aumento da Inativação Intracelular da Droga por Moléculas Contendo Tióis

A cisplatina pode ser inativada por inúmeros constituintes citoplasmáticos, incluindo a abundante molécula nucleofílica glutationa (GSH) e as metalotioneínas ricas em cisteínas. Essas reações de inativação podem ser espontâneas, devido à alta reatividade da molécula de ciplatina após reagir com a água no meio intracelular, ou catalisadas por enzimas, como glutationa S-transferases e sistema de peroxiredoxinas (Pedersen et al., 2009; Tew, 1994).

As metalotioneínas são ricas em cisteínas contendo grupos tióis, os quais são centros de interação ideais para a cisplatina. Por essa razão, não é inesperado que aumentos nos níveis celulares de metalotioneínas tenham sido observados em modelos celulares de resistência à ciplatina. Da mesma maneira, concentrações elevadas de moléculas contendo tiol produzem resistência por diminuir os níveis de agente antitumoral disponível para interação com o DNA. O aumento no conteúdo de GSH foi demonstrado em vários modelos tumorais de resistência à cisplatina, e confirmados em estudos clínicos. Elevações na quantidade de GSH intracelular podem ocorrer como resultado de um aumento de expressão do gene de γ-glutamilcisteíno sintetases (γ-GCS), cujo produto é uma enzima limitante na biossintese de GSH (Siddik, 2003).

A reação de conjugação da ciplatina com GSH pode ser catalisada pela glutationa S-transferase π (GSTπ), a qual é membro de uma família de enzimas envolvidas nas reações de detoxificação de xenobióticos. O aumento na expressão de GSTπ, junto com elevados níveis de GSH em células tumorais resistentes, sugerem que a inativação enzimática da cisplatina contribui significativamente para o fenótipo em nível clínico e o aumento nas reações de conjugação entre GSH e cisplatina são geralmente aceitas como um fator significante na resistência (Siddik, 2003).

3.3 Aumento da Eficácia dos Mecanismos de Reparo ao DNA

A formação e a persistência dos adutos resultantes da interação entre a molécula de cisplatina ativada com o DNA são vitais na indução do efeito citotóxico da droga, o que culminará na apoptose da célula tumoral. Dessa forma, um aumento na taxa de reparo desses adutos atenuaria o processo apoptótico. A demonstração de que um aumento na taxa de reparo está associado com uma inibição da citotoxicidade induzida pela droga em muitas linhagens de tumores murinos e humanos suporta essa ideia. Como todos os outros mecanismos, o reparo não está universalmente presente em todas as linhagens resistentes à cisplatina. Quando presente, entretanto, a contribuição do aumento no reparo para a resistência é relativamente baixo, e usualmente resulta num acréscimo de resistência na ordem de 1,5-2,0 vezes. Este aumento limitado é, de qualquer forma, considerado como significante uma vez que a inativação dos adutos é em grande parte devido ao reparo do DNA. Esse limite na capacidade de reparo na resistência é apoiado pelo achado de que o aumento do

reparo permanece num platô, mesmo quando a resistência à ciplatina aumenta progressivamente em protocolos de exposição crônica à droga (Siddik, 2003; Siddik et al., 1998).

O sistema de reparo por excisão de nucleotídeos, ou NER (do inglês *nucleotide excision repair*), é a principal via para a remoção do dano ao DNA gerado pela ciplatina. A significância desse sistema de reparo é demonstrada pelos achados que defeitos celulares nessa via resultam numa hipersensibilidade à cisplatina, e a restauração desse sistema restabelece a sensibilidade a níveis normais. O sistema NER tem ampla especificidade, e não são observadas diferenças na excisão de adutos induzidos por cisplatina e outras drogas a base de platina. De fato, um aumento no reparo dos adutos nas células resistentes também se aplica a outros análogos de platina, o que sugere que esse mecanismo de resistência talvez seja difícil de ser superado para agentes antineoplásicos baseados em platina (Chaney and Sancar, 1996; Siddik, 2003).

Apesar de o sistema NER ser composto de pelo menos 17 diferentes proteínas, parece que *upregulation* de somente algumas proteínas-chave é necessário para aumentar a capacidade de reparo em células tumorais. Dentre as principais proteínas descritas estão ERCC1 ou o complexo ERCC1/XPF e XPA. Pelo contrário, células de tumor testicular sensíveis à cisplatina expressam níveis muito baixos dessas proteínas (Siddik, 2003).

Além do NER, o sistema de reparo de mau pareamento, ou MMR (do inglês *mismatch repair*) também parece estar envolvido na resistência à ciplatina, pois vários estudos apontam a deficiência nesse mecanismo de reparo como um fator indutor desse processo. Entretanto, esse sistema atua no reconhecimento de dano, apresentando um papel crítico na manutenção da integridade do genoma durante a replicação, e não participa ativamente no reparo de adutos de cisplatina. Uma hipótese proposta é de que o MMR reconhece a lesão causada pela droga e inicia a cascata de indução de apoptose. Dessa forma, se houver um defeito ou perda de eficácia desse sistema, o sinal de apoptose mediado pelo MMR deixa de existir e isso confere a resistência observada (Fink et al., 1998; Martin et al., 2008). Além disso, para manter a estabilidade genômica, é vital que o reparo do DNA ocorra antes da replicação. Contudo, a resistência advém quando as células aumentam sua capacidade de replicar o DNA passando o aduto e, então, iniciando o reparo no período pós-replicação. Isto aumenta a habilidade das células tumorais de tolerar

altos níveis de DNA danificado induzido por cisplatina. A este respeito, é significativo que o desvio na replicação é aumentado de 3-6 vezes por defeitos nas proteínas hMLH1 ou hMSH6, as quais apresentam grande importância no papel do MMR na resistência à cisplatina, uma vez elas fazem parte do sistema MMR (Chaney and Sancar, 1996; Martin et al., 2008; Siddik, 2003).

3.4 Outros Mecanismos de Resistência Propostos

3.4.1 Aumento do Importe Nuclear de Proteínas

Além dos três mecanismos descritos acima, alguns outros processos descritos na literatura parecem estar relacionados à aquisição de resistência. O primeiro refere-se ao transporte nuclear aumentado de proteínas envolvidas no reparo ao DNA (Kinoshita et al., 2012). O transporte de proteínas do citoplasma para o núcleo e vice-versa ocorre através do complexo de poro nuclear (NPC, do inglês *nuclear pore complex*), o qual é formado por cerca de 30 nucleoporinas, algumas das quais possuem sítios de ligação com carioporinas, ou importinas. Importinas formam uma superfamília de proteínas que reconhecem sequências de localização nuclear (NLS, do inglês, *nuclear localization signal*), geralmente grupos de resíduos de aminoácidos ácidos, e então medeiam o trânsito para o núcleo. O complexo importina α/β é um complexo chave na via de importe nuclear em eucariotos e esse mecanismo é usualmente referido como importe nuclear clássico. Uma segunda via de transporte nuclear ocorre quando proteínas que não possuem NLS formam complexos com outra que o possui e, dessa maneira, podem ser importadas. Ainda não está claro se esse mecanismo de co-importe é um sistema de reserva ou se ele é essencial para a regulação do importe nuclear *in vivo*. De qualquer maneira, até agora, o importe via co-importe só foi observado para proteínas que funcionam em complexos bem definidos durante o reparo por excisão. Finalmente, outras rotas de transporte, referidas como mecanismos de transporte alternativos e os quais independem de importinas, também já foi identificado. Dados sugerem que proteínas que utilizam a rota alternativa possam ligar-se diretamente com nucleoporinas e serem transportadas (Knudsen et al., 2009; Sorokin et al., 2007).

3.4.2 Cofilina-1

Outros trabalhos na literatura propõem o envolvimento da proteína cofilina-1 na resistência à cisplatina (Castro et al., 2010). A família de proteínas ADF/cofilina é expressa em todas as células eucarióticas e em três formas nos mamíferos: ADF (*actin-depolymerizing factor*), cofilina-1 (a principal forma nos tecidos não-musculares) e cofilina-2 (a principal forma no músculo). A cofilina-1 é uma proteína ubíqua de aproximadamente 19 kDa que é capaz de ligar-se tanto a actina-G (monomérica) quanto a actina-F (filamentosa), sendo um reconhecido fator de regulação dinâmica de polimerização e despolimerização dos filamentos de actina (Wang et al., 2007). Além das suas propriedades reguladoras da dinâmica de citoesqueleto, a cofilina-1 apresenta outras funções celulares. Uma delas é a capacidade de translocar para o núcleo após alguns estímulos químicos ou físicos. Essa translocação nuclear somente ocorre se a proteína estiver no seu estado ativado, ou seja, defosforilado, uma vez que somente nesse estado a cofilina-1 expõe a sua sequência de sinalização nuclear. Ainda, estudos mostraram que a cofilina-1 apresenta a função de carrear actina consigo para o núcleo (Pendleton et al., 2003). Por outro lado, estudos de proteômica mostraram que a cofilina-1 foi encontrada translocando para a mitocôndria após indução de apoptose por estaurosporina em células de neuroblastoma e, em linfomas, a oxidação da cofilina-1 também promove sua translocação para a mitocôndria e morte celular programada (Chua et al., 2003; Klamt et al., 2009).

Portanto, a resistência aos agentes alquilantes pode surgir de diversas associações e rotas intracelulares. O que é notável é que uma única via dificilmente será a exclusiva responsável por esse fenômeno e, por essa razão, o estudo de uma única rota intracelular parece ser insuficiente para se chegar a uma conclusão sobre os mecanismos de resistência desenvolvidos pelas células tumorais a estes fármacos.

II. Artigo Científico

Cisplatin resistance signature in non-small cells lung cancer involves the *CFL1*-associated gene network

Marco A. De Bastiani[1], Matheus Becker[1], Mauro Antônio A. Castro[1], Fábio Klamt[1]

Authors' Affiliation: [1] Laboratory 24, Department of Biochemistry, ICBS/UFRGS, Porto Alegre/RS, Brazil, Ramiro Barcelos 2600 - Anexo, Rio Branco.
Corresponding Author: Marco A. De Bastiani; Phone – 555184523814 / 555132375021; E-mail – tyrev@hotmail.com; Laboratory 24, Department of Biochemistry, ICBS/UFRGS, Porto Alegre/RS, Brazil, Rua Ramiro Barcelos 2600 - Anexo, Rio Branco.

Abstract

Lung cancer is one of major causes of cancer-related deaths in the world and non-small cell lung cancer is the most frequently diagnosed type of lung cancer. The main clinical interventions are therapies based on alkylating agents such as cisplatin. Therefore, resistance to cisplatin poses as a major obstacle in the treatment of these patients. Early studies showed that the *CFL1* gene product cofilin-1 might be associated with cisplatin resistance in non-small cells lung cancer. In this study we employ a bioinformatic approach based on microarray data analysis to investigate this hypothesis, and for that, five microarray datasets (three tumor cell lines datasets – two containing different cell line panels and one including an acute treatment with cisplatin – and two tissue biopsies datasets – one comprising tumor *versus* control samples and one deriving from early stages non-small cell lung cancer patients) were utilized. We observed that the *CFL1*-associated genes behaved similarly to the major mechanisms of cisplatin resistance cited in literature and that it also responded to cisplatin treatment together with these mechanisms in cell lines models. Likewise, tissue biopsies demonstrated elevated *CFL1*-associated genes in tumor cells, as well as the other mechanisms compared. In especial, our results showed a tight correlation between *CFL1*-associated gene activity and excision repair mechanism of resistance both in cellular models and tissue microarray data. Altogether, our findings suggest that the *CFL1*-associated network might play a role in cisplatin resistance as well as in the non-small cells lung cancer development and treatment.

Keywords: Non-small cells lung cancer, cisplatin resistance, cofilin, microarray.

Highlights

CFL1-associated network follows similar pattern of gene activation as NER network in alkylating agents resistant cell lines and after acute treatment of NSCLC cell line with cisplatin, which suggests that its role in chemoresistance might be by contribution to NER pathway.

Correlation analysis suggests that *CFL1*-associated network presents differential contribution to drug resistance depending on histological type and alkylating agent utilized.

Increased *CFL1*-associated network gene activation in patients with low overall survival in stage I of NSCLC supports the use of this network in predictive evaluation of recently diagnosed NSCLC patients.

1. INTRODUCTION

Lung cancer is one of the most insidious malignant tumors in oncology, ranking among the top causes of cancer-related deaths and showing high prevalence in both sexes (Jemal et al., 2011). Approximately 80-85% of all lung cancers are non-small cell lung cancer (NSCLC), which include squamous cell carcinoma, adenocarcinoma, and large-cell carcinoma. For these patients, chemotherapy forms the foundation of their treatment and is critical in determining their survival and quality of life. Currently, the platinum-based therapy with ciplatin, carboplatin or oxaliplatin is the mainstay of chemotherapy for NSCLC and is usually given in combination with other agents (Ramalingam and Belani, 2008). Unfortunately, despite these therapies, the disease is rarely curable and prognosis is poor, with overall 5-year survival rate of only 15%.

Several factors contribute to such low survival rate and one of the most common includes tumor cell resistance (chemoresistance) to cisplatin. Chemoresistance may be innate or acquired and may apply to a single agent or to a class of agents with same or similar antineoplasic mechanisms of action (Chang, 2011; Siddik, 2003; Torigoe et al., 2005). The literature describes three major mechanisms of resistance to cisplatin. The first includes increased efflux, associated with membrane proteins, notably multidrug resistance (MDR) and multidrug

resistance protein (MRP), of the ATP binding cassette (ABC) transporter family encoding efflux pumps, and copper-transporting ATPases (Dmitriev, 2011; Kuo, 2009; Parker et al., 1991). The second mechanism includes intracellular drug inactivation via metallothioneins (Pedersen et al., 2009) and glutathione-related enzymes systems (Laborde, 2010; Tew, 1994). Cisplatin exerts its cytotoxic effect by disrupting the DNA macromolecule, mainly through the formation of intra-strand adducts and inter-strand crosslinks that are repaired through the nucleotide excision repair (NER) pathway (Wang and Lippard, 2005). Therefore, the third well established mechanism of chemoresistance to cisplatin is enhanced repair system machinery. It is also postulated that tumors that are defective in mismatch repair (MMR) pathway also become more resistant to cisplatin than their MMR-proficient counterparts (Rosell et al., 2002). Moreover, since these repair pathways take place in the nucleus, all proteins necessary for these repair activities must be translocated to this compartment. For that reason, nuclear translocation presents a supplementary mechanism for regulation of DNA repair activity (Knudsen et al., 2009) and play an important role in the chemoresistance (Kinoshita et al., 2012). In addition, deregulation of nuclear transport is implicated in the mislocalization and altered function of a variety of proteins, including tumor suppressors, which can have dire cellular consequences and potentially lead to the initiation and progression of cancer (Fabbro and Henderson, 2003).

Although each of the mechanisms mentioned may play an important role in the chemoresistance to cisplatin, it is important to realize the multifactorial nature of the drug resistance. In other words, it is possible that one tumor cell utilizes a combination of two of those mechanisms or even the three of them, even if the majority of the resistance effects are distributed unequally among these systems. Therefore, when studying chemoresistance, it is important to evaluate the status and the contribution of all the mechanisms. This approach is essential to identify which process is responsible for the final effect or even to search for possible new players that may be leading or contributing to the development of the drug resistance phenotype. However, to deal with the high complexity of biological processes, bioinformatic tools and high-throughput methodologies, such as microarray, have become important experimental approaches (Lonning et al., 2007).

Previous studies of our group analyzed the relation between drug toxicity of 118 chemotherapy agents withthe gene expression patternsof six NSCLC cell lines of

the NCI-60 human tumor cell line anticancer drug screen reported the possible involvement of cofilin-1 in the resistance to alkylating agents (Castro et al., 2010). Cofilin-1 is the product of *CFL1* gene and a member of the ADF/cofilin family of proteins, classically involved in the regulation of the actin cytoskeleton dynamic. In recent years, however, several other functions have been attributed to cofilin-1, such as its nuclear translocation under cellular stress, nuclear translocation of actin and induction of apoptosis via mitochondrial translocation (Chua et al., 2003; Klamt et al., 2009; Pendleton et al., 2003). In this study, we aim to explore the role of cofilin-1 in the chemoresistance to cisplatin by analyzing the *CFL1*-associated gene network expression together with the gene network of other mechanisms of resistance in five microarray datasets of NSCLC extracted from the Gene Expression Omnibus public repository.

2. MATERIALS AND METHODS

2.1 Microarray Datasets

The microarray data was extracted from the Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo/) utilizing the key words "cisplatin" or "alkylating agents" and "non-small cell lung cancer". Three datasets of interest were chosen and analyzed: GEO ID: GSE4127; GSE6410; GSE11117; and GSE14814. In addition, we utilized the NCI-60 cancer cell panel (GEO ID: GSE5846). When available, the datasets were downloaded as raw data and normalized using the Affymetrix® Expression Console™ software (v.1.1).

2.2 Construction of the Networks

The genes for NER and MMR networks were extracted from the pathways database of the Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/pathway.html) in Kegg map: hsa03420; and Kegg map: hsa03430, respectively. The genes selected for the Inactivation/Efflux and Nuclei Import networks were curated from the literature (Dmitriev, 2011; Gelain et al., 2009; Knudsen et al., 2009; Siddik, 2003; Teng et al., 2006). The *CFL1* interaction network was extracted from Castro et al (Castro et al., 2010).

After the gene selection, the network was built utilizing the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) version 9.0 (http://string-

db.org/). All genes collected served as entry for the database and filtered for "experiments", "medium confidence", plus one layer of interaction. Genes that did not form interactions were excluded and each mechanism was grouped manually using the Medusa interface. The genes added by the second layer of interaction were grouped and termed "Linkers", for they helped unite the network. The resultant network is presented in figure 1.

2.3 Gene Expression Network Analysis and Statistics

For the gene expression analysis we utilized the ViaComplex software version 1.0 (Castro et al., 2009). The main advantage of this program is that it is able to distribute a given quantity (quantitative or qualitative data) onto gene/protein interaction networks. To do this, ViaComplex overlaps functional information (e.g. microarray data) with interaction information (supplied by the gene network built).

We utilized statistical analysis available in the ViaComplex software which estimates the relative expression level of Groups of Functionally Associated Genes (GFAGs) and is describe elsewhere (Castro et al., 2007). Briefly, to obtain a quantitative parameter that characterizes the functional state of each Group of Functionally Associated Genes (GFAG) in the sample, ViaComplex measures the information content using Shannon's entropy. We display the data of activity derived from the comparisons. Unless otherwise mentioned, the analyses of diversity of the comparisons were non-significant.

Some additional analyses of correlation were made using Pearson correlation statistics. 50% growth inhibition values of cisplatin and carboplatin were obtained in the Mechanism of Action drug activity database of the National Cancer Institute Developmental Therapeutics Program (http://discover.nci.nih.gov/datasetsNature2000.jsp) and http://www.biomedcentral.com/content/supplementary/1471-2407-6-174-S1.doc provided by Gemma et al (Gemma et al., 2006).

3. RESULTS

3.1 Tumor cell lines datasets

In a recent study, Castro et al. analyzed the correlation between gene expression and drug toxicity (GI50 values) of six NSCLC cell lines extracted from

the NCI-60 panel and observed that high levels of *CFL1* mRNA were correlated with resistance against different anticancer drugs—mainly alkylating agents (Castro et al., 2010). Here we evaluated our resistance network utilizing the GSE4127 dataset, which contains gene expression of 29 lung cancer cell lines (10 NSCLC adenocarcinomas, 9 NSCLC squamous cells carcinomas and 10 small cells). The authors also provide the GI50 values of cisplatin in each cell line (Gemma et al., 2006). In figure 2A, we utilized the ViaComplex software to investigate the gene expression relation between the cell line with higher GI50 *versus* the cell line with lower GI50 values. There was significant increase in the expression of the *CFL1* cluster, as well as in the NER, inactivation/efflux and linkers clusters. However, we observed no correlation when the cell lines where grouped according to histological type and then analyzed (data not shown).

We also investigated the role of each gene of our network by analyzing the Pearson correlation between gene expression and cisplatin GI50 for all 99 genes. For that, we analyzed the NCI-60 tumor cell line panel and altogether the two datasets comprised 25 NSCLC cell lines (15 adenocarcinomas and 10 squamous cells carcinomas). 17 genes showed significant Pearson correlation when all cell lines were analyzed. When grouped by histological type, we found significant correlation for 13 genes for adenocarcinomas and 8 genes for squamous cells carcinomas (see appendix A for complete data).

The cisplatin derivate carboplatin is also an important antineoplasic agent frequently utilized in clinic due to its milder side effects. Carboplatin share similar mechanism of action with cisplatin, and cross-resistance between these two drugs is a well observed phenomena. Hence, to strengthen the relevance of the data obtained for cisplatin, we performed the Pearson correlations of gene expression of our network *versus* carboplatin GI50. As expected, most of the genes that showed correlation in the cisplatin analysis also showed correlation for carboplatin (see appendix B for complete data). Surprisingly, the number of genes exhibiting significant correlation in the carboplatin analysis was higher than in the cisplatin analysis. This might suggest that, although both agents share common resistance mechanisms, the groups of genes analyzed appear to play different contributions to the level of resistance against each drug. When the cell lines were grouped by histological type, the results displayed similar pattern, which implies that histological type also accounts for different resistance outcome. Nevertheless, some genes appear

to play special roles in this context, as they were commonly observed for both drugs and both histological types (see appendix C for details). These data are summarized in Figure 2B.

So far, our results showed that the most resistant cell lines to ciplatin display enhanced gene expression of the *CFL1* associated group and mostly NER group. If these processes are important to the intrinsic or acquired resistance, it is reasonable to think that ciplatin insult will promote increase in gene expression of these groups. To test this assumption, we utilized the GEO dataset GSE6410, in which gene expression changes in response to acute cisplatin treatment were analyzed by microarray technology in A549 NSCLC cells. Cells were treated with 50 μM of cisplatin (or drug-free media) for 1 hour and incubated for a further 10 hours in drug-free media before the cisplatin-induced gene expression changes were investigated. Control and cisplatin-treated samples were collected from three independent experiments (Almeida et al., 2008). Once again, the *CFL1* associated genes showed significant increase in expression, along with NER cluster (figure 3). Changes in gene expression of linkers, Nuclei Import and inactivation/efflux gene groups did not reach statistical significance.

3.2 Tumor biopsies datasets

We next aimed to evaluate a possible clinical relevance for the *CFL1* associated gene network. To our knowledge, there is no GEO dataset with microarray data of biopsies of NSCLC patients resistant to cisplatin or alkylating agents. Hence, we utilized the GSE11117 and GSE14814 datasets to analyze our network. In GSE11117 dataset, tissue samples from a series of 41 chemotherapy-naïve non-small cell lung cancer patients and 15 control patients with inflammatory lung diseases were obtained during routine clinical procedures and gene expression profiles were obtained using a highly sensitive oligonucleotide array platform (Baty et al., 2010). For our analysis, we utilized only patients with adenocarcinoma or squamous cells carcinoma compared to the control samples provided. For both histological types, tissue microarray data showed increased activity of *CFL1* associated genes and NER cluster (figure 4). For the comparison of adenocarcinoma *versus* control, the inactivation/efflux group also displayed increased expression (figure 4A), and for the squamous cells carcinoma *versus* control comparison, the linkers group exhibited significant statistical increase (figure 4B). However, the

increase observed in the linkers cluster did not appear to be homogeneous throughout the group (data not shown).

Recent clinical trials have led to the adoption of adjuvant cisplatin-based chemotherapy for patients with resected stages IB to IIIA NSCLC. The 5-year survival advantage conferred by this approach in these studies ranged from 4% in the International Adjuvant Lung Trial to 15% in National Cancer Institute of Canada Clinical Trials Group JBR.10 (Pisters et al., 2007; Winton et al., 2005). Therefore, we next aimed to investigate the role of *CFL1* associated genes in early stages of NSCLC utilizing the GSE14814 dataset, which provides gene expression profiling of mRNA isolated from frozen JBR.10 tumor samples (Zhu et al., 2010). We first divided the patients by histological type then analyzed those with adenocarcinoma and squamous cells carcinoma by Pearson correlation of overall survival (OS) *versus* expression of each gene of our network (see appendix D and E for detailed data). Our results show a different pattern of correlation between the two histological types, with adenocarcinoma displaying greater response of *CFL1* network in both stages I and II.

In the International Adjuvant Lung Trial and the National Cancer Institute of Canada Clinical Trials Group JBR.10, the application of cisplatin-based chemotherapy has been controversial for patients in stage I, as neither showed a significant survival benefit in stage IB and a potential detrimental effect was observed in stage IA. Thus, we examined a possible involvement of *CFL1* associated genes with these outcomes. For this, we grouped the adenocarcinoma and squamous cells carcinoma patients in lower-50 and upper-50 values of OS, and then, utilizing the ViaComplex statistical analysis, we compared lower-50 patients *versus* upper-50 patients. As presented in table 1, the results showed that for both histological types, the lower-50 patients, those presenting poorer outcome, displayed increased activity of the *CFL1*-associated genes. In addition, the adenocarcinoma group also showed increased activity of the inactivation/efflux cluster.

4. Discussion

The use of tumor cell lines has become a powerful tool of investigation in oncology, increasing our understanding of several molecular mechanisms and pathways in cancer (Gazdar et al., 2010; van Staveren et al., 2009; Voskoglou-Nomikos et al., 2003). To explore the potential role of *CFL1*-associated network in

the cellular resistance to cisplatin, we first investigated the differences in gene expression of cisplatin resistant lung cancer cell line in comparison to drug sensible cell line. The results showed increased expression of NER and inactivation/efflux clusters, two well described processes involved in chemoresistance. Similarly, the *CFL1*-associated network also displayed increased gene activity, which might suggest its involvement in resistances mechanisms. The lack of significance in MMR and nuclei import gene groups is not surprising, as it is expected that cells do not necessarily display all mechanisms at the same time (Perez, 1998). When acute response to cisplatin treatment was investigated utilizing a dataset of treated and untreated NSCLC cell line, *CFL1*-associated genes again showed enhanced activity together with NER related gene. These results might imply that *CFL1*-associated network could be somewhat contributing with NER system. The translocation of cofilin-1 with actin to the nucleus has been described by Pendleton and colls as a requirement for certain stress-induced responses; however other possible roles of cofilin-1 in the nuclear context might be possible (Pendleton et al., 2003).

We also evaluated the possible role of each gene in the resistance context of NSCLC by investigating the Pearson correlation between cisplatin or carboplatin GI50 *versus* gene expression in adenocarcinomas and squamous cells carcinomas. The different set of gene expression related with the increasing resistance to these drugs suggests that, although sharing the same resistance group, distinct genes might be playing special roles in the resistance to one drug and not to the other. For instance, the gene *MAP3K5* – a member of MAP kinases family associated with cascades of cellular responses evoked by changes in the environment and mediating cell survival, differentiation and apoptosis – appears correlated with carboplatin drug response and not cisplatin; the same happens to some members of ERCC family and *RAD23A*, involved in NER function. Furthermore, disparities in histological type should be taken into account when analyzing chemoresistance, as the faction of genes regulated also varies. When observe the genes that show correlation for both drugs, but consider the histological type, we see several members of the GTF2H family of transcription factors, *ERCC3*, *TPI*, and others, displaying correlation in adenocarcinomas but not in squamous cells carcinomas. Nevertheless, our results show that some genes might play key roles in this context, as they appeared to be common to both alkylating agents and histological type. Unsurprisingly, such general genes are included in NER, MMR and inactivation/efflux mechanisms. Contrarily,

CFL1-associated genes seem to follow a somewhat histological and/or agent-specific involvement in the chemoresistance context.

Although cell line models are a recognized instrument in molecular oncology studies, the fact that they fail to express the totality of the tumor microenvironment is a major bias that should be taken into account (van Staveren et al., 2009). For that reason, we also investigated the clinical importance of *CFL1*-associated genes utilizing NSCLC biopsies datasets. When NSCLC patients with adenocarcinoma or squamous cells carcinoma were compared to control patients, once again we observed increased gene activity of NER group together with *CFL1*-associated cluster. In accordance with the cell line models, *CFL1* network seems to be activated together with NER cluster in the tumor tissue when compared to control tissue. In addition, we again found the differential histological context influencing the processes active in our network, as observed by the increased expression of the inactivation/efflux group in adenocarcinoma, but not in squamous cells carcinoma. It is important to point out at this point that the control group provided was patients with inflammatory lung diseases. The inflammatory context of the tumor is a well recognized modulatory factor to the tumoral development and the attenuation of this variable here might bias the real circumstance regulating our network as a whole (Coussens and Werb, 2002; Demaria et al., 2010). Nonetheless, the consistency of our findings and the place of this dataset in the midground between the total lack of tumoral context observed in cell lines models and the full complexity displayed *in vivo* support the possible involvement of *CFL1* network in tumor development.

Recent clinical trials have re-confirmed the efficacy of alkylating agents-based chemotherapies in early stages NSCLC patients, especially for adjuvant cisplatin-based combinations. However, for stage I patients, the adoption of these treatments did not show significant overall survival alterations, but a potential detrimental effect was observed (Muller et al., 2011; Pisters et al., 2007; Winton et al., 2005). Thus, we aimed to investigate the role of *CFL1*-associated network in early stages of NSCLC. Following the pattern of histological influence observed so far in our results, we analyzed the Pearson correlation between overall survival *versus* gene expression of stage I and II patients with adenocarcinoma and squamous cells carcinoma separately. Difference in gene activation pattern also varied according to histological type in both stages. However, conversely to the cell line model, tumor biopsies showed greater response of our network for the

adenocarcinoma group, not for squamous cells carcinomas, in both stage I and II. This discrepancy might be a reflection of a tumor context absent in cellular models.

Finally, when we divided stage I patients with NSCLC in lower-50 and upper-50 according to overall survival rate and compared their gene expression, we observed that for both histological types the *CFL1*-associated cluster showed increased activity. This result suggests that these genes may be somewhat associated with lower survival. Indeed, in a recent study, Muller et al. reported that early stage NSCLC patients presenting higher expression of cofilin-1 displayed poorer clinical outcome (Muller et al., 2011). Although we did not find correlation significance for the *CFL1* gene, our data support a possible involvement of its network in this scenario. Moreover, the enhance activity of *CFL1*-associated group in stage I patients and the debate over the possible inefficiency of cisplatin-based therapies for these patients could mean that these genes are playing some role in the resistance to cisplatin. Additionally, according to our results, this effect might be specially accentuated in patients with adenocarcinoma. In this case, the use of alkylating agents-based therapies should be re-evaluated as a first line intervention for these patients. Therefore, further studies are necessary to fully understand the involvement of cofilin-1 and its associating partners in the development of NSCLC resistance to cisplatin and also the possible clinical application of this in prognostic and treatment.

5. Conclusion

In summary, our results point out a possible involvement of *CFL1*-associated genes in cisplatin resistance in non-small cell lung cancer. We also observed that the increased activity of these genes occurred together with enhanced activity of NER-related network, suggesting that the *CFL1* group contribution to chemoresistance may be happening via NER pathway. Additionally, cell line models showed that the participation of *CFL1* network was different according to histological type, fact that was further supported by biopsies microarrays. Finally, tissue datasets also demonstrated that increased in *CFL1*-associated genes could be involved in lower survival and in alkylating agents chemotherapy inefficacy of NSCLC patients.

REFERENCES

Almeida, G.M., Duarte, T.L., Farmer, P.B., Steward, W.P., Jones, G.D., 2008. Multiple end-point analysis reveals cisplatin damage tolerance to be a chemoresistance mechanism in a NSCLC model: implications for predictive testing. International journal of cancer. Journal international du cancer 122, 1810-1819.

Baty, F., Facompre, M., Kaiser, S., Schumacher, M., Pless, M., Bubendorf, L., Savic, S., Marrer, E., Budach, W., Buess, M., Kehren, J., Tamm, M., Brutsche, M.H., 2010. Gene profiling of clinical routine biopsies and prediction of survival in non-small cell lung cancer. American journal of respiratory and critical care medicine 181, 181-188.

Castro, M.A., Dal-Pizzol, F., Zdanov, S., Soares, M., Muller, C.B., Lopes, F.M., Zanotto-Filho, A., da Cruz Fernandes, M., Moreira, J.C., Shacter, E., Klamt, F., 2010. *CFL1* expression levels as a prognostic and drug resistance marker in nonsmall cell lung cancer. Cancer 116, 3645-3655.

Castro, M.A., Filho, J.L., Dalmolin, R.J., Sinigaglia, M., Moreira, J.C., Mombach, J.C., de Almeida, R.M., 2009. ViaComplex: software for landscape analysis of gene expression networks in genomic context. Bioinformatics 25, 1468-1469.

Castro, M.A., Mombach, J.C., de Almeida, R.M., Moreira, J.C., 2007. Impaired expression of NER gene network in sporadic solid tumors. Nucleic acids research 35, 1859-1867.

Chang, A., 2011. Chemotherapy, chemoresistance and the changing treatment landscape for NSCLC. Lung Cancer 71, 3-10.

Chua, B.T., Volbracht, C., Tan, K.O., Li, R., Yu, V.C., Li, P., 2003. Mitochondrial translocation of cofilin is an early step in apoptosis induction. Nature cell biology 5, 1083-1089.

Coussens, L.M., Werb, Z., 2002. Inflammation and cancer. Nature 420, 860-867.

Demaria, S., Pikarsky, E., Karin, M., Coussens, L.M., Chen, Y.C., El-Omar, E.M., Trinchieri, G., Dubinett, S.M., Mao, J.T., Szabo, E., Krieg, A., Weiner, G.J., Fox, B.A., Coukos, G., Wang, E., Abraham, R.T., Carbone, M., Lotze, M.T., 2010. Cancer and inflammation: promise for biologic therapy. J Immunother 33, 335-351.

Dmitriev, O.Y., 2011. Mechanism of tumor resistance to cisplatin mediated by the copper transporter ATP7B. Biochemistry and cell biology = Biochimie et biologie cellulaire 89, 138-147.

Fabbro, M., Henderson, B.R., 2003. Regulation of tumor suppressors by nuclear-cytoplasmic shuttling. Experimental cell research 282, 59-69.

Gazdar, A.F., Gao, B., Minna, J.D., 2010. Lung cancer cell lines: Useless artifacts or invaluable tools for medical science? Lung Cancer 68, 309-318.

Gemma, A., Li, C., Sugiyama, Y., Matsuda, K., Seike, Y., Kosaihira, S., Minegishi, Y., Noro, R., Nara, M., Seike, M., Yoshimura, A., Shionoya, A., Kawakami, A., Ogawa, N., Uesaka, H., Kudoh, S., 2006. Anticancer drug clustering in lung cancer based on gene expression profiles and sensitivity database. BMC cancer 6, 174.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., Forman, D., 2011. Global cancer statistics. CA: a cancer journal for clinicians 61, 69-90.

Kinoshita, Y., Kalir, T., Rahaman, J., Dottino, P., Kohtz, D.S., 2012. Alterations in nuclear pore architecture allow cancer cell entry into or exit from drug-resistant dormancy. The American journal of pathology 180, 375-389.

Klamt, F., Zdanov, S., Levine, R.L., Pariser, A., Zhang, Y., Zhang, B., Yu, L.R., Veenstra, T.D., Shacter, E., 2009. Oxidant-induced apoptosis is mediated by oxidation of the actin-regulatory protein cofilin. Nature cell biology 11, 1241-1246.

Knudsen, N.O., Andersen, S.D., Lutzen, A., Nielsen, F.C., Rasmussen, L.J., 2009. Nuclear translocation contributes to regulation of DNA excision repair activities. DNA repair 8, 682-689.

Kuo, M.T., 2009. Redox regulation of multidrug resistance in cancer chemotherapy: molecular mechanisms and therapeutic opportunities. Antioxidants & redox signaling 11, 99-133.

Laborde, E., 2010. Glutathione transferases as mediators of signaling pathways involved in cell proliferation and cell death. Cell death and differentiation 17, 1373-1380.

Lonning, P.E., Knappskog, S., Staalesen, V., Chrisanthar, R., Lillehaug, J.R., 2007. Breast cancer prognostication and prediction in the postgenomic era. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 18, 1293-1306.

Muller, C.B., de Barros, R.L., Castro, M.A., Lopes, F.M., Meurer, R.T., Roehe, A., Mazzini, G., Ulbrich-Kulczynski, J.M., Dal-Pizzol, F., Fernandes, M.C., Moreira, J.C., Xavier, L.L., Klamt, F., 2011. Validation of cofilin-1 as a biomarker in non-small cell lung cancer: application of quantitative method in a retrospective cohort. Journal of cancer research and clinical oncology 137, 1309-1316.

Parker, R.J., Eastman, A., Bostick-Bruton, F., Reed, E., 1991. Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin-DNA lesions and reduced drug accumulation. The Journal of clinical investigation 87, 772-777.

Pedersen, M.O., Larsen, A., Stoltenberg, M., Penkowa, M., 2009. The role of metallothionein in oncogenesis and cancer prognosis. Progress in histochemistry and cytochemistry 44, 29-64.

Pendleton, A., Pope, B., Weeds, A., Koffer, A., 2003. Latrunculin B or ATP depletion induces cofilin-dependent translocation of actin into nuclei of mast cells. The Journal of biological chemistry 278, 14394-14400.

Perez, R.P., 1998. Cellular and molecular determinants of cisplatin resistance. Eur J Cancer 34, 1535-1542.

Pisters, K.M., Evans, W.K., Azzoli, C.G., Kris, M.G., Smith, C.A., Desch, C.E., Somerfield, M.R., Brouwers, M.C., Darling, G., Ellis, P.M., Gaspar, L.E., Pass, H.I., Spigel, D.R., Strawn, J.R., Ung, Y.C., Shepherd, F.A., 2007. Cancer Care Ontario and American Society of Clinical Oncology adjuvant chemotherapy and adjuvant radiation therapy for stages I-IIIA resectable non small-cell lung cancer guideline. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 25, 5506-5518.

Rosell, R., Lord, R.V., Taron, M., Reguart, N., 2002. DNA repair and cisplatin resistance in non-small-cell lung cancer. Lung Cancer 38, 217-227.

Siddik, Z.H., 2003. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 22, 7265-7279.

Teng, S.C., Wu, K.J., Tseng, S.F., Wong, C.W., Kao, L., 2006. Importin KPNA2, NBS1, DNA repair and tumorigenesis. Journal of molecular histology 37, 293-299.

Tew, K.D., 1994. Glutathione-associated enzymes in anticancer drug resistance. Cancer research 54, 4313-4320.

Torigoe, T., Izumi, H., Ishiguchi, H., Yoshida, Y., Tanabe, M., Yoshida, T., Igarashi, T., Niina, I., Wakasugi, T., Imaizumi, T., Momii, Y., Kuwano, M., Kohno, K., 2005. Cisplatin resistance and transcription factors. Current medicinal chemistry. Anti-cancer agents 5, 15-27.

van Staveren, W.C., Solis, D.Y., Hebrant, A., Detours, V., Dumont, J.E., Maenhaut, C., 2009. Human cancer cell lines: Experimental models for cancer cells in situ? For cancer stem cells? Biochimica et biophysica acta 1795, 92-103.

Voskoglou-Nomikos, T., Pater, J.L., Seymour, L., 2003. Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. Clinical cancer research : an official journal of the American Association for Cancer Research 9, 4227-4239.

Winton, T., Livingston, R., Johnson, D., Rigas, J., Johnston, M., Butts, C., Cormier, Y., Goss, G., Inculet, R., Vallieres, E., Fry, W., Bethune, D., Ayoub, J., Ding, K., Seymour, L., Graham, B., Tsao, M.S., Gandara, D., Kesler, K., Demmy, T., Shepherd, F., 2005. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. The New England journal of medicine 352, 2589-2597.

Zhu, C.Q., Ding, K., Strumpf, D., Weir, B.A., Meyerson, M., Pennell, N., Thomas, R.K., Naoki, K., Ladd-Acosta, C., Liu, N., Pintilie, M., Der, S., Seymour, L., Jurisica, I., Shepherd, F.A., Tsao, M.S., 2010. Prognostic and predictive gene signature for adjuvant chemotherapy in resected non-small-cell lung cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 28, 4417-4424.

FIGURES/TABLES AND LEGENDS

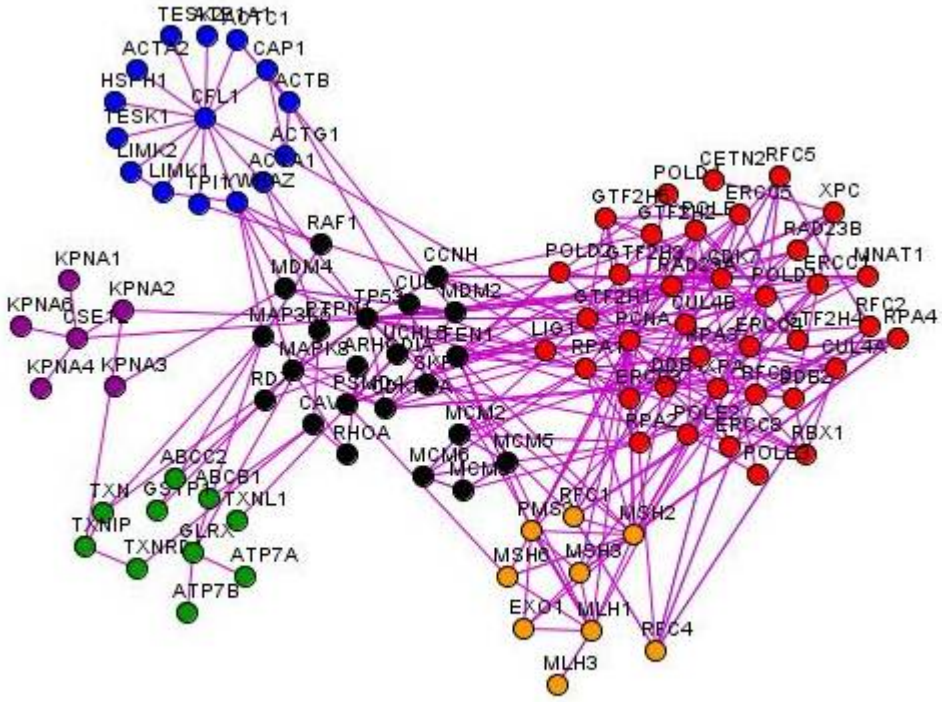
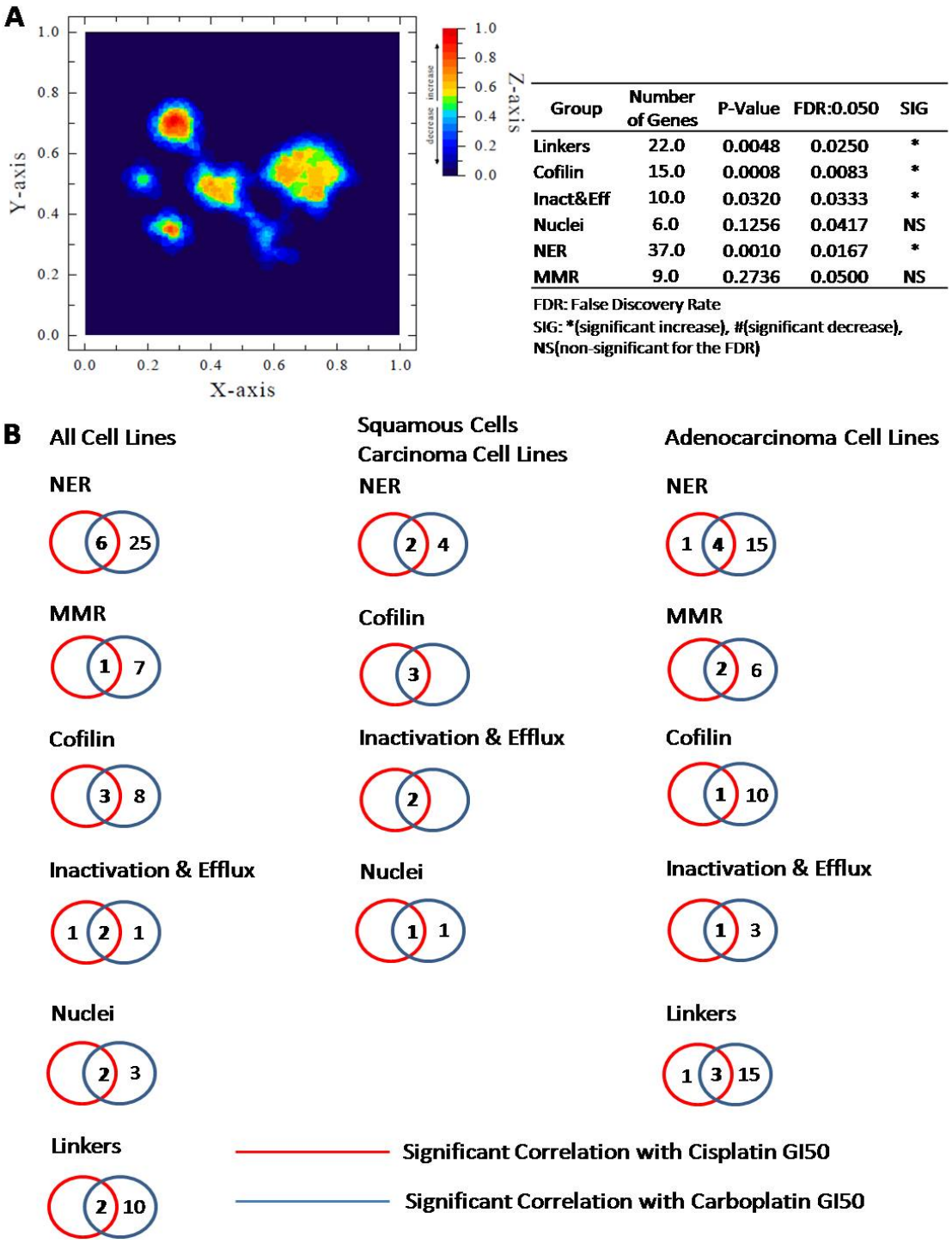


Figure 1. Alkylating drug resistance network utilized for topological analysis with ViaComplex software. Blue cluster represents the *CFL1* associated genes; red cluster represents NER genes; orange cluster represents MMR genes; green cluster represents inactivation/efflux genes; purple cluster represents nuclei import associated genes; and black cluster represents linkers genes.

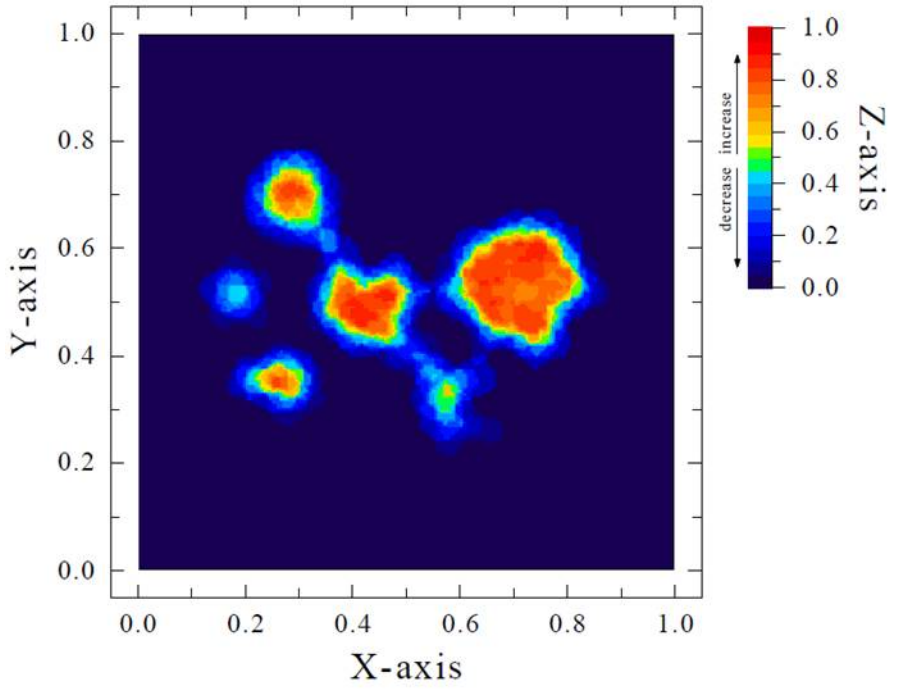


Group	Number of Genes	P-Value	FDR:0.050	SIG
Linkers	22.0	0.0048	0.0250	*
Cofilin	15.0	0.0008	0.0083	*
Inact&Eff	10.0	0.0320	0.0333	*
Nuclei	6.0	0.1256	0.0417	NS
NER	37.0	0.0010	0.0167	*
MMR	9.0	0.2736	0.0500	NS

FDR: False Discovery Rate
SIG: *(significant increase), #(significant decrease), NS(non-significant for the FDR)

Figure 2. (A) Two-state landscape analysis of gene/protein interaction networks. Coordinates (X- and Y-axis) represent normalized values of the input network topology. Color gradient (Z-axis) represents the relative functional state mapped onto graph according to the data input (higher GI50 cell line-a *vs.* lower GI50 cell line-b), where z=a/(a+b), e.g., a is greater than b when z>0.55 (yellow to red), lower than b when z<0.45 (cyan to blue) and equivalent to b when 0.45<z<0.55 (green). The

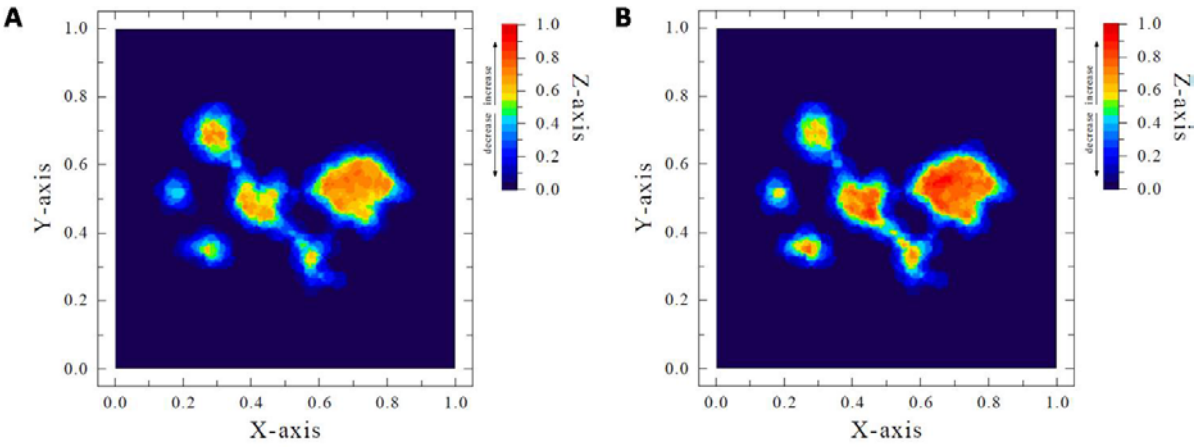
landscape is generated by ViaComplex V1.0 with the following options: plot as "3D-Graph", build on "node", resolution "level-50", contrast "level-50", smoothness "level-50" and zoom "level-50". Statistical analysis was generated with the options “bootstrap” on “5000” and “FDR cutoff” on “0.05”; diversity data was non-significant for all groups. (B) Number of genes with significant Pearson correlation of ciplatin or carboplatin GI50 *versus* gene expression.



Group	Number of Genes	P-Value	FDR:0.050	SIG
Linkers	22.0	0.0984	0.0417	NS
Cofilin	15.0	0.0124	0.0167	*
Inact&Eff	10.0	0.0706	0.0333	NS
Nuclei	6.0	0.0668	0.0250	NS
NER	37.0	0.0012	0.0083	*
MMR	9.0	0.3356	0.0500	NS

FDR: False Discovery Rate
SIG: *(significant increase), #(significant decrease), NS(non-significant for the FDR)

Figure 3. Two-state landscape analysis of gene/protein interaction networks. Coordinates (X- and Y-axis) represent normalized values of the input network topology. Color gradient (Z-axis) represents the relative functional state mapped onto graph according to the data input (A549 cells treated with 50 uM cisplatin-a *vs.* untreated A549 cells-b), where z=a/(a+b), e.g., a is greater than b when z>0.55 (yellow to red), lower than b when z<0.45 (cyan to blue) and equivalent to b when 0.45<z<0.55 (green). The landscape is generated by ViaComplex V1.0 with the following options: plot as "3D-Graph", build on "node", resolution "level-50", contrast "level-50", smoothness "level-50" and zoom "level-50". Statistical analysis was generated with the options “bootstrap” on “5000” and “FDR cutoff” on “0.05”; diversity data was non-significant for all groups.



Group	Number of Genes	P-Value	FDR:0.050	SIG
Linkers	22.0	0.1184	0.0333	NS
Cofilin	15.0	0.0022	0.0250	*
Inact&Eff	10.0	0.0002	0.0083	*
Nuclei	6.0	0.3038	0.0417	NS
NER	37.0	0.0022	0.0250	*
MMR	9.0	0.4904	0.0500	NS

FDR: False Discovery Rate

SIG: *(significant increase), #(significant decrease), NS(non-significant for the FDR)

Group	Number of Genes	P-Value	FDR:0.050	SIG
Linkers	22.0	0.0108	0.0250	*
Cofilin	15.0	0.0052	0.0167	*
Inact&Eff	10.0	0.0568	0.0333	NS
Nuclei	6.0	0.1466	0.0417	NS
NER	37.0	0.0036	0.0083	*
MMR	9.0	0.2642	0.0500	NS

FDR: False Discovery Rate

SIG: *(significant increase), #(significant decrease), NS(non-significant for the FDR)

Figure 4. Two-state landscape analysis of gene/protein interaction networks. Coordinates (X- and Y-axis) represent normalized values of the input network topology. Color gradient (Z-axis) represents the relative functional state mapped onto graph according to the data input (experiment-a *vs.* control-b), where z=a/(a+b), e.g., a is greater than b when z>0.55 (yellow to red), lower than b when z<0.45 (cyan to blue) and equivalent to b when 0.45<z<0.55 (green). The landscape is generated by ViaComplex V1.0 with the following options: plot as "3D-Graph", build on "node", resolution "level-50", contrast "level-50", smoothness "level-50" and zoom "level-50". Statistical analysis was generated with the options “bootstrap” on “5000” and “FDR cutoff” on “0.05”; diversity data was non-significant for all groups, except the linkers group in the squamous cells carcinoma *versus* control comparison. (A) Comparison between adenocarcinoma *versus* control. (B) Comparison between squamous cells carcinoma *versus* control.

Table 1. Lower50 OS *versus* upper50 OS comparison in stage I NSCLC patients

		Adenocarcinoma			Squamous cells carcinoma		
Group	Number of Genes	P-Value	FDR:0.050	SIG	P-Value	FDR:0.050	SIG
Linkers	22.0	0.2440	0.0417	NS	0.1308	0.0333	NS
Cofilin	15.0	0.0001	0.0083	*	0.0001	0.0083	*
Inact&Eff	10.0	0.0444	0.0167	NS	0.0340	0.0167	NS
Nuclei	6.0	0.0646	0.0250	NS	0.2896	0.0417	NS
NER	37.0	0.1468	0.0333	NS	0.1070	0.0250	NS
MMR	9.0	0.4446	0.0500	NS	0.3590	0.0500	NS

FDR: False Discovery Rate

SIG: *(significant increase), #(significant dencrease), NS(non-significant for the FDR)

APPENDICES

Appendix A

CISPLATIN GI50 *versus* GENE EXPRESSION CORRELATION ANALYSIS						
All Cell Lines						
Cluster	Number of Genes	Number of Significant Genes	Gene Symbol	Pearson R	R^2	*Pvalue* (two-tailed)
Linkers	22	2	CUL1	0,4234	0,1793	0,0349
			PSMD4	0,7216	0,5208	< 0.0001
NER	37	6	RAD23B	0,3979	0,1583	0,0489
			GTF2H4	0,5808	0,3373	0,0023
			RPA1	0,4954	0,2455	0,0118
			ERCC8	0,5053	0,2553	0,01
			ERCC3	0,5856	0,3429	0,0021
			ERCC4	0,5055	0,2555	0,0099
MMR	9	1	MSH6	0,4241	0,1799	0,0346
Nuclei Import	6	2	KPNA6	0,459	0,2107	0,021
			KPNA1	0,5122	0,2623	0,0089
Inactivation & Efflux	10	3	ATP7A	0,8793	0,7731	< 0.0001
			GSTP1	0,5408	0,2925	0,0052
			ABCC2	0,616	0,3795	0,001
Cofilin	15	3	ACTG1	0,4844	0,2347	0,0141
			TESK1	0,7857	0,6173	< 0.0001
			YWHAZ	0,4516	0,204	0,0234

Squamous Cells Carcinoma Cell Lines						
Cluster	Number of Genes	Number of Significant Genes	Gene Symbol	Pearson R	R^2	*Pvalue* (two-tailed)
Linkers	22	0	--	--	--	--
NER	37	2	RAD23B	0,7023	0,4932	0,0236
			RPA1	0,6615	0,4375	0,0373
MMR	9	0	--	--	--	--
Nuclei Import	6	1	KPNA1	0,8201	0,6725	0,0037
Inactivation & Efflux	10	2	ATP7A	0,9431	0,8894	< 0.0001
			ABCC2	0,9103	0,8286	0,0003
Cofilin	15	3	ACTG1	0,711	0,5056	0,0212
			TESK1	0,9241	0,854	0,0001
			YWHAZ	0,7392	0,5463	0,0146

Adenocarcinoma Cell Lines						
Cluster	Number of Genes	Number of Significant Genes	Gene Symbol	Pearson R	R^2	*Pvalue* (two-tailed)
Linkers	22	4	CUL1	0,5454	0,2974	0,0355
			PSMD4	0,9105	0,8289	< 0.0001

			RAF1	0,5246	0,2752	0,0447
			ARHGDIA	0,5597	0,3132	0,0301
NER	37	5	POLD2	0,605	0,366	0,0169
			GTF2H4	0,6658	0,4433	0,0067
			GTF2H5	0,8451	0,7142	< 0.0001
			GTF2H2	0,5892	0,3471	0,0208
			ERCC3	0,8301	0,6891	0,0001
MMR	9	2	MSH6	0,5792	0,3355	0,0237
			MSH2	0,5542	0,3071	0,0321
Nuclei Import	6	0	--	--	--	--
Inactivation & Efflux	10	1	ATP7A	0,7686	0,5907	0,0008
Cofilin	15	1	TPI1	0,6184	0,3825	0,014

Appendix B

CARBOPLATIN GI50 *versus* GENE EXPRESSION CORRELATION ANALYSIS						
All Cell Lines						
Cluster	Number of Genes	Number of Significant Genes	Gene Symbol	Pearson R	R^2	*Pvalue* (two-tailed)
Linkers	22	12	MAP3K5	0,553	0,3059	0,0041
			CUL1	0,5622	0,316	0,0034
			MAPK8	0,6517	0,4247	0,0004
			SKP1	0,4158	0,1729	0,0387
			PSMD4	0,5963	0,3555	0,0017
			TP53	0,4065	0,1653	0,0437
			MCM5	0,4803	0,2307	0,0151
			UCHL5	0,5873	0,3449	0,002
			MDM4	0,6351	0,4033	0,0006
			PTPN1	0,5904	0,3486	0,0019
			RHOA	0,512	0,2621	0,0089
			RDX	0,4536	0,2058	0,0228
NER	37	31	LIG1	0,7228	0,5224	< 0.0001
			POLE	0,4223	0,1784	0,0355
			RFC5	0,506	0,2561	0,0099
			RFC3	0,5315	0,2825	0,0062
			RFC2	0,4407	0,1942	0,0275
			RAD23B	0,6364	0,405	0,0006
			RAD23A	0,6228	0,3878	0,0009
			RBX1	0,4928	0,2429	0,0123
			POLE2	0,5008	0,2508	0,0108
			POLE3	0,4547	0,2068	0,0224
			POLD1	0,7057	0,498	< 0.0001
			POLD2	0,5128	0,2629	0,0088
			PCNA	0,4891	0,2393	0,0131
			DDB1	0,6974	0,4863	0,0001
			GTF2H4	0,7509	0,5638	< 0.0001
			GTF2H3	0,4701	0,221	0,0177
			GTF2H5	0,5184	0,2687	0,0079
			GTF2H2	0,5084	0,2585	0,0095
			GTF2H1	0,6065	0,3679	0,0013
			CETN2	0,5738	0,3293	0,0027
			RPA4	0,5567	0,3099	0,0038
			RPA3	0,1964	0,03856	0,3468
			RPA1	0,625	0,3906	0,0008
			ERCC8	0,7136	0,5093	< 0.0001
			RPA2	0,6326	0,4002	0,0007
			ERCC5	0,4056	0,1645	0,0442
			ERCC3	0,5363	0,2876	0,0057

			ERCC4	0,618	0,382	0,001
			ERCC1	0,5127	0,2629	0,0088
			MNAT1	0,4029	0,1624	0,0458
			CUL4A	0,4134	0,1709	0,04
			CUL4B	0,4038	0,1631	0,0453
MMR	9	8	PMS2	0,4599	0,2115	0,0207
			RFC1	0,4851	0,2353	0,014
			MLH1	0,504	0,2541	0,0102
			MLH3	0,645	0,416	0,0005
			EXO1	0,4222	0,1782	0,0355
			MSH6	0,6211	0,3857	0,0009
			MSH3	0,7941	0,6305	< 0.0001
			MSH2	0,4574	0,2092	0,0215
Nuclei Import	6	5	CSE1L	0,5152	0,0084	0,2654
			KPNA6	0,8143	< 0.0001	0,6631
			KPNA3	0,465	0,0192	0,2162
			KPNA2	0,6011	0,0015	0,3613
			KPNA1	0,7912	< 0.0001	0,6259
Inactivation & Efflux	10	3	TXNL1	0,5282	0,279	0,0066
			ATP7A	0,6191	0,3833	0,001
			ABCC2	0,4646	0,2158	0,0193
Cofilin	15	11	ACTG1	0,7423	0,551	< 0.0001
			TESK1	0,6566	0,4311	0,0004
			TESK2	0,5046	0,2547	0,0101
			TPI1	0,5087	0,2588	0,0094
			HSPH1	0,4549	0,2069	0,0223
			CAP1	0,431	0,1857	0,0315
			ACTA1	0,4392	0,1929	0,028
			LIMK1	0,4676	0,2186	0,0184
			CFL1	0,5278	0,2786	0,0067
			YWHAZ	0,735	0,5402	< 0.0001
			ATP1A1	0,5061	0,2561	0,0098

Squamous Cells Carcinoma Cell Lines						
Cluster	Number of Genes	Number of Significant Genes	Gene Symbol	Pearson R	R^2	*Pvalue* (two-tailed)
Linkers	22	2	MAP3K5	0,6983	0,4876	0,0247
			CDKN1A	-0,6629	0,4395	0,0367
NER	37	6	RAD23B	0,6393	0,4088	0,0466
			RAD23A	0,6423	0,4125	0,0452
			GTF2H4	0,7954	0,6327	0,0059
			RPA1	0,6467	0,4182	0,0433
			ERCC4	0,6521	0,4253	0,041
			ERCC1	0,7525	0,5663	0,012

MMR	9	0	--	--	--	--
Nuclei Import	6	2	KPNA6	0,7241	0,5243	0,0179
			KPNA1	0,8412	0,7076	0,0023
Inactivation & Efflux	10	2	ATP7A	0,8962	0,8032	0,0004
			ABCC2	0,8074	0,652	0,0047
Cofilin	15	3	ACTG1	0,7291	0,5317	0,0167
			TESK1	0,8039	0,6463	0,0051
			YWHAZ	0,6774	0,4588	0,0314

Adenocarcinoma Cell Lines						
Cluster	Number of Genes	Number of Significant Genes	Gene Symbol	Pearson R	R^2	*Pvalue* (two-tailed)
Linkers	22	18	MAP3K5	0,5372	0,2886	0,0389
			CUL1	0,8198	0,6721	0,0002
			MAPK8	0,8352	0,6976	0,0001
			SKP1	0,7625	0,5814	0,0009
			PSMD4	0,6636	0,4404	0,007
			CCNH	0,5612	0,3149	0,0295
			MCM2	0,7022	0,4931	0,0035
			MCM3	0,5589	0,3124	0,0303
			MCM5	0,6067	0,368	0,0165
			MCM6	0,5581	0,3115	0,0306
			UCHL5	0,6898	0,4759	0,0044
			MDM2	0,8907	0,7933	< 0.0001
			MDM4	0,7739	0,599	0,0007
			FEN1	0,5884	0,3462	0,021
			RAF1	0,644	0,4148	0,0096
			PTPN1	0,8512	0,7246	< 0.0001
			RHOA	0,8182	0,6695	0,0002
			RDX	0,5719	0,3271	0,0259
NER	37	19	GTF2H4	0,7517	0,5651	0,0012
			GTF2H3	0,7046	0,4964	0,0034
			GTF2H5	0,7079	0,5011	0,0031
			CDK7	0,5545	0,3075	0,0319
			GTF2H2	0,5657	0,32	0,028
			GTF2H1	0,7388	0,5458	0,0017
			XPA	0,9144	0,8362	< 0.0001
			CETN2	0,6983	0,4877	0,0038
			RPA4	0,8547	0,7304	< 0.0001
			RPA3	0,5214	0,2719	0,0462
			RPA1	0,7128	0,5081	0,0029
			ERCC8	0,8704	0,7576	< 0.0001
			RPA2	0,773	0,5975	0,0007
			ERCC5	0,6838	0,4676	0,0049
			ERCC3	0,6985	0,488	0,0038
			ERCC4	0,8959	0,8027	< 0.0001

			MNAT1	0,6492	0,4214	0,0088
			CUL4A	0,8837	0,781	< 0.0001
			CUL4B	0,5728	0,3281	0,0256
MMR	9	8	PMS2	0,7287	0,0021	0,5311
			RFC1	0,8086	0,0003	0,6538
			MLH1	0,675	0,0058	0,4557
			MLH3	0,8708	< 0.0001	0,7583
			EXO1	0,6246	0,0128	0,3902
			MSH6	0,7821	0,0006	0,6116
			MSH3	0,8998	< 0.0001	0,8097
			MSH2	0,7255	0,0022	0,5264
Nuclei Import	6	6	CSE1L	0,671	0,4503	0,0062
			KPNA6	0,8826	0,779	< 0.0001
			KPNA4	0,6273	0,3935	0,0123
			KPNA3	0,5504	0,3029	0,0335
			KPNA2	0,6756	0,4564	0,0057
			KPNA1	0,8195	0,6716	0,0002
Inactivation & Efflux	10	4	TXNL1	0,625	0,3906	0,0127
			ATP7A	0,7622	0,5809	0,001
			TXN	0,5408	0,2925	0,0374
			TXNIP	0,5295	0,2804	0,0424
Cofilin	15	11	ACTG1	0,7721	0,5961	0,0007
			TESK1	0,9164	0,8398	< 0.0001
			TESK2	0,6444	0,4152	0,0095
			TPI1	0,7011	0,4916	0,0036
			HSPH1	0,6258	0,3916	0,0126
			CAP1	0,515	0,2652	0,0495
			ACTA1	0,7228	0,5224	0,0023
			LIMK1	0,6852	0,4694	0,0048
			CFL1	0,8198	0,6721	0,0002
			YWHAZ	0,7804	0,609	0,0006
			ATP1A1	0,5958	0,355	0,0191
			ACTB	0,4971	0,2471	0,0594

Appendix C

GENES WITH SIGNIFICANT PEARSON CORRELATION IN COMMON FOR CIPLATIN AND CARBOPLATIN			
All Cell Lines			
Group	Gene Symbol	HUGO Approved Name	UniProt Function
NER	RAD23B	RAD23 homolog B	Plays a central role both in proteosomal degradation of misfolded proteins and DNA repair. In DNA repair, it is involved in DNA excision repair by stabilizing XPC protein and may play a part in DNA damage recognition and/or in altering chromatin structure to allow access by damage-processing enzymes.
	GTF2H4	general transcription factor IIH, polypeptide 4, 52kDa	Component of the core-TFIIH basal transcription factor involved in nucleotide excision repair (NER) of DNA and, when complexed to CAK, in RNA transcription by RNA polymerase II.
	RPA1	replication protein A1, 70kDa	Plays an essential role in several cellular processes in DNA metabolism including replication, recombination and DNA repair. Binds and subsequently stabilizes single-stranded DNA intermediates and thus prevents complementary DNA from reannealing.
	ERCC8	excision repair cross-complementing rodent repair deficiency, complementation group 8	Substrate-recognition component of the CSA complex, a DCX (DDB1-CUL4-X-box) E3 ubiquitin-protein ligase complex, involved in transcription-coupled nucleotide excision repair. It is required for the recruitment of XAB2, HMGN1 and TCEA1/TFIIS to a transcription-coupled repair complex which removes RNA polymerase II-blocking lesions from the transcribed strand of active genes.
	ERCC3	excision repair cross-complementing rodent repair deficiency, complementation group 3	ATP-dependent 3'-5' DNA helicase, component of the core-TFIIH basal transcription factor, involved in nucleotide excision repair (NER) of DNA and, when complexed to CAK, in RNA transcription by RNA polymerase II. Acts by opening DNA either around the RNA transcription start site or the DNA damage.
	ERCC4	excision repair cross-complementing rodent repair deficiency, complementation group 4	Structure-specific DNA repair endonuclease responsible for the 5-prime incision during DNA repair. Involved in homologous recombination that assists in removing interstrand cross-link.
MMR	MSH6	mutS homolog 6	Component of the post-replicative DNA mismatch repair system (MMR). When bound, MutS alpha bends the DNA helix and shields approximately 20 base pairs, and recognizes single base mismatches and dinucleotide insertion-deletion loops (IDL) in the DNA. After mismatch binding, forms a ternary complex with the MutL alpha heterodimer, which is

			thought to be responsible for directing the downstream MMR events, including strand discrimination, excision, and resynthesis.
Cofilin	ACTG1	actin, gamma 1	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.
	TESK1	testis-specific kinase 1	Dual specificity protein kinase activity catalyzing autophosphorylation and phosphorylation of exogenous substrates on both serine/threonine and tyrosine residues.
	YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.
Inactivation and Efflux	ATP7A	ATPase, Cu++ transporting, alpha polypeptide	May supply copper to copper-requiring proteins within the secretory pathway, when localized in the trans-Golgi network. Under conditions of elevated extracellular copper, it relocalized to the plasma membrane where it functions in the efflux of copper from cells.
	ABCC2	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	Mediates hepatobiliary excretion of numerous organic anions. May function as a cellular cisplatin transporter.
Nuclei Import	KPNA6	karyopherin alpha 6 (importin alpha 7)	Functions in nuclear protein import as an adapter protein for nuclear receptor KPNB1. Binds specifically and directly to substrates containing either a simple or bipartite NLS motif.
	KPNA1	karyopherin alpha 1 (importin alpha 5)	Functions in nuclear protein import as an adapter protein for nuclear receptor KPNB1. Binds specifically and directly to substrates containing either a simple or bipartite NLS motif.
Linkers	CUL1	cullin 1	Core component of multiple cullin-RING-based SCF (SKP1-CUL1-F-box protein) E3 ubiquitin-protein ligase complexes, which mediate the ubiquitination of proteins involved in cell cycle progression, signal transduction and transcription.
	PSMD4	proteasome (prosome, macropain) 26S subunit, non-ATPase, 4	Binds and presumably selects ubiquitin-conjugates for destruction. Displays selectivity for longer polyubiquitin chains.
Squamous Cells Carcinoma Cell Lines			
Group	Gene Symbol	HUGO Approved Name	UniProt Function
NER	RAD23B	RAD23 homolog B	Plays a central role both in proteosomal degradation of misfolded proteins and DNA repair. In DNA repair, it is involved in DNA excision repair by stabilizing XPC protein and may play a part in DNA

			damage recognition and/or in altering chromatin structure to allow access by damage-processing enzymes.
	RPA1	replication protein A1, 70kDa	Plays an essential role in several cellular processes in DNA metabolism including replication, recombination and DNA repair. Binds and subsequently stabilizes single-stranded DNA intermediates and thus prevents complementary DNA from reannealing.
Cofilin	ACTG1	actin, gamma 1	Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.
	TESK1	testis-specific kinase 1	Dual specificity protein kinase activity catalyzing autophosphorylation and phosphorylation of exogenous substrates on both serine/threonine and tyrosine residues.
	YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.
Inactivation and Efflux	ATP7A	ATPase, Cu++ transporting, alpha polypeptide	May supply copper to copper-requiring proteins within the secretory pathway, when localized in the trans-Golgi network. Under conditions of elevated extracellular copper, it relocalized to the plasma membrane where it functions in the efflux of copper from cells.
	ABCC2	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	Mediates hepatobiliary excretion of numerous organic anions. May function as a cellular cisplatin transporter.
Nuclei Import	KPNA1	karyopherin alpha 1 (importin alpha 5)	Functions in nuclear protein import as an adapter protein for nuclear receptor KPNB1. Binds specifically and directly to substrates containing either a simple or bipartite NLS motif.
Adenoarcinoma Cell Lines			
Group	Gene Symbol	HUGO Approved Name	UniProt Function
NER	GTF2H4	general transcription factor IIH, polypeptide 4, 52kDa	Component of the core-TFIIH basal transcription factor involved in nucleotide excision repair (NER) of DNA and, when complexed to CAK, in RNA transcription by RNA polymerase II.
	GTF2H5	general transcription factor IIH, polypeptide 5	Component of the TFIIH basal transcription factor involved in nucleotide excision repair (NER) of DNA and, when complexed to CAK, in RNA transcription by RNA polymerase II. Necessary for the stability of the TFIIH complex and for the presence of normal levels of TFIIH in the cell.

	GTF2H2	general transcription factor IIH, polypeptide 2, 44kDa	Component of the core-TFIIH basal transcription factor involved in nucleotide excision repair (NER) of DNA and, when complexed to CAK, in RNA transcription by RNA polymerase II. The N-terminus interacts with and regulates XPD whereas an intact C-terminus is required for a successful escape of RNAP II form the promoter.
	ERCC3	excision repair cross-complementing rodent repair deficiency, complementation group 3	ATP-dependent 3'-5' DNA helicase, component of the core-TFIIH basal transcription factor, involved in nucleotide excision repair (NER) of DNA and, when complexed to CAK, in RNA transcription by RNA polymerase II. Acts by opening DNA either around the RNA transcription start site or the DNA damage.
MMR	MSH6	mutS homolog 6	Component of the post-replicative DNA mismatch repair system (MMR). When bound, MutS alpha bends the DNA helix and shields approximately 20 base pairs, and recognizes single base mismatches and dinucleotide insertion-deletion loops (IDL) in the DNA. After mismatch binding, forms a ternary complex with the MutL alpha heterodimer, which is thought to be responsible for directing the downstream MMR events, including strand discrimination, excision, and resynthesis.
	MSH2	mutS homolog 2, colon cancer, nonpolyposis type 1	Component of the post-replicative DNA mismatch repair system (MMR). Forms two different heterodimers: MutS alpha (MSH2-MSH6 heterodimer) and MutS beta (MSH2-MSH3 heterodimer) which binds to DNA mismatches thereby initiating DNA repair.
Cofilin	TPI1	triosephosphate isomerase 1	Belongs to the triosephosphate isomerase family. Defects in TPI1 are the cause of triosephosphate isomerase deficiency (TPI deficiency), an autosomal recessive disorder which is the most severe clinical disorder of glycolysis.
Inactivation and Efflux	ATP7A	ATPase, Cu++ transporting, alpha polypeptide	May supply copper to copper-requiring proteins within the secretory pathway, when localized in the trans-Golgi network. Under conditions of elevated extracellular copper, it relocalized to the plasma membrane where it functions in the efflux of copper from cells.
Linkers	CUL1	cullin 1	Core component of multiple cullin-RING-based SCF (SKP1-CUL1-F-box protein) E3 ubiquitin-protein ligase complexes, which mediate the ubiquitination of proteins involved in cell cycle progression, signal transduction and transcription.
	PSMD4	proteasome (prosome, macropain) 26S subunit, non-ATPase, 4	Binds and presumably selects ubiquitin-conjugates for destruction. Displays selectivity for longer polyubiquitin chains.
	RAF1	v-raf-1 murine leukemia viral oncogene homolog 1	Serine/threonine-protein kinase that acts as a regulatory link between the membrane-associated

			Ras GTPases and the MAPK/ERK cascade, and this critical regulatory link functions as a switch determining cell fate decisions including proliferation, differentiation, apoptosis, survival and oncogenic transformation. RAF1 activation initiates a mitogen-activated protein kinase (MAPK) cascade that comprises a sequential phosphorylation of the dual-specific MAPK kinases (MAP2K1/MEK1 and MAP2K2/MEK2) and the extracellular signal-regulated kinases (MAPK3/ERK1 and MAPK1/ERK2).

Appendix D

ADENOCARCINOMA OVERALL SURVIVAL *versus* GENE EXPRESSION CORRELATION ANALYSIS						
Stage I						
Cluster	Number of Genes	Number of Significant Genes	Gene Symbol	Pearson R	R^2	*Pvalue* (two-tailed)
Linkers	22	0	--	--	--	--
NER	37	3	PCNA	0,5452	0,2972	0,0438
			GTF2H5	0,7525	0,5662	0,0019
			CDK7	0,5637	0,3177	0,0358
MMR	9	1	RFC4	0,536	0,2873	0,0482
Nuclei Import	6	0	--	--	--	--
Inactivation & Efflux	10	1	TXNIP	-0,5762	0,332	0,031
Cofilin	15	2	TPI1	-0,5617	0,3155	0,0366
			LIMK1	-0,5241	0,2747	0,0544

Stage II						
Cluster	Number of Genes	Number of Significant Genes	Gene Symbol	Pearson R	R^2	*Pvalue* (two-tailed)
Linkers	22	2	FEN1	0,6116	0,3741	0,0201
			RHOA	0,5359	0,2871	0,0483
NER	37	6	RFC2	0,658	0,433	0,0105
			RAD23A	0,5645	0,3187	0,0355
			POLE3	0,6115	0,3739	0,0201
			GTF2H1	-0,6554	0,4296	0,0109
			DDB2	-0,5351	0,2863	0,0486
			RPA1	0,5221	0,2726	0,0555
			RPA4	-0,5907	0,3489	0,0261
MMR	9	0	--	--	--	--
Nuclei Import	6	1	KPNA2	0,5462	0,2984	0,0433
Inactivation & Efflux	10	1	ATP7B	-0,6417	0,4118	0,0134
Cofilin	15	0	--	--	--	--

Appendix E

SQUAMOUS CELLS CARCINOMA OVERALL SURVIVAL *versus* GENE EXPRESSION CORRELATION ANALYSIS						
Stage I						
Cluster	Number of Genes	Number of Significant Genes	Gene Symbol	Pearson R	R^2	*Pvalue* (two-tailed)
Linkers	22	0	--	--	--	--
NER	37	3	POLD1	-0,3984	0,1587	0,0485
			GTF2H4	-0,4098	0,168	0,0419
			RPA3	-0,4681	0,2191	0,0183
MMR	9	0	--	--	--	--
Nuclei Import	6	0	--	--	--	--
Inactivation & Efflux	10	1	ABCC2	-0,4565	0,2084	0,0218
Cofilin	15	1	HSPH1	0,5367	0,288	0,0057

Stage II						
Cluster	Number of Genes	Number of Significant Genes	Gene Symbol	Pearson R	R^2	*Pvalue* (two-tailed)
Linkers	22	0	--	--	--	--
NER	37	0	--	--	--	--
MMR	9	0	--	--	--	--
Nuclei Import	6	0	--	--	--	--
Inactivation & Efflux	10	0	--	--	--	--
Cofilin	15	0	--	--	--	--

III. Conclusões e Perspectivas

Nesse trabalho, demonstramos que a rede de interação gênica da cofilina-1 pode estar envolvida com a resistência a agentes alquilantes – entre eles a cisplatina - em câncer de pulmão de não-pequenas células (CPNPC). Ainda, nossos resultados também sugerem que a contribuição dos genes relacionados à cofilina-1 na resistência está possivelmente relacionada com a via de reparo por excisão de nucleotídeos, já que esses dois grupos comportaram-se similarmente na maioria das análises realizadas. Além disso, ainda que a atividade gênica dos mesmos mecanismos de resistência tenha aumentado, pudemos observar variações nos padrões de correlação de acordo com o tipo histológico e com o agente alquilante, o que sugere que há uma contribuição diferencial dos genes estudados na quimioresistência. Ademais, nossa análise utilizando microarranjos de biópsias está de acordo com a hipótese de que o aumento da atividade da rede da cofilina-1 está relacionado com desfecho ruim para pacientes em estágios iniciais de CPNPC. Entretanto, mais estudos são necessários para investigar os mecanismos pelos quais o grupo de genes relacionados à cofilina-1 está influenciando a resistência à cisplatina. Dessa maneira, as perspectivas desse trabalho envolvem avaliar o papel da cofilina-1 e/ou de sua rede em comparação com outros mecanismos descritos de resistência à cisplatina em um modelo celular utilizando linhagens de CPNPC. Para isso, poder-se-á utilizar protocolos de indução de resistência (adquirida ou intrínseca), realizar ensaios que avaliem os diferentes processos de quimioresistência e então comparar células controles não resistentes com células resistentes. Outra oportunidade interessante de se explorar nesse modelo é a possível relação entre rede da cofilina-1 com rede NER. Indícios da interação entre esses dois grupos podem ser investigados ao se estudar a compartimentalização da cofilina-1 durante o processo de aquisição de resistência, visto que esta proteína apresenta uma sequência de sinalização nuclear. Finalmente, maiores indicativos da importância dessa proteína nesse contexto poderão ser testados ao se super-expressar e silenciar a cofilina-1, e/ou genes relacionados a ela.

Referências Bibliográficas

Almeida, G.M., Duarte, T.L., Farmer, P.B., Steward, W.P., Jones, G.D., 2008. Multiple end-point analysis reveals cisplatin damage tolerance to be a chemoresistance mechanism in a NSCLC model: implications for predictive testing. International journal of cancer. Journal international du cancer 122, 1810-1819.

Arriagada, R., Bergman, B., Dunant, A., Le Chevalier, T., Pignon, J.P., Vansteenkiste, J., 2004. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. The New England journal of medicine 350, 351-360.

Baty, F., Facompre, M., Kaiser, S., Schumacher, M., Pless, M., Bubendorf, L., Savic, S., Marrer, E., Budach, W., Buess, M., Kehren, J., Tamm, M., Brutsche, M.H., 2010. Gene profiling of clinical routine biopsies and prediction of survival in non-small cell lung cancer. American journal of respiratory and critical care medicine 181, 181-188.

Castro, M.A., Dal-Pizzol, F., Zdanov, S., Soares, M., Muller, C.B., Lopes, F.M., Zanotto-Filho, A., da Cruz Fernandes, M., Moreira, J.C., Shacter, E., Klamt, F., 2010. CFL1 expression levels as a prognostic and drug resistance marker in nonsmall cell lung cancer. Cancer 116, 3645-3655.

Castro, M.A., Filho, J.L., Dalmolin, R.J., Sinigaglia, M., Moreira, J.C., Mombach, J.C., de Almeida, R.M., 2009. ViaComplex: software for landscape analysis of gene expression networks in genomic context. Bioinformatics 25, 1468-1469.

Castro, M.A., Mombach, J.C., de Almeida, R.M., Moreira, J.C., 2007. Impaired expression of NER gene network in sporadic solid tumors. Nucleic acids research 35, 1859-1867.

Chaney, S.G., Sancar, A., 1996. DNA repair: enzymatic mechanisms and relevance to drug response. Journal of the National Cancer Institute 88, 1346-1360.

Chang, A., 2011. Chemotherapy, chemoresistance and the changing treatment landscape for NSCLC. Lung Cancer 71, 3-10.

Chua, B.T., Volbracht, C., Tan, K.O., Li, R., Yu, V.C., Li, P., 2003. Mitochondrial translocation of cofilin is an early step in apoptosis induction. Nature cell biology 5, 1083-1089.

Coussens, L.M., Werb, Z., 2002. Inflammation and cancer. Nature 420, 860-867.

Demaria, S., Pikarsky, E., Karin, M., Coussens, L.M., Chen, Y.C., El-Omar, E.M., Trinchieri, G., Dubinett, S.M., Mao, J.T., Szabo, E., Krieg, A., Weiner, G.J., Fox, B.A., Coukos, G., Wang, E., Abraham, R.T., Carbone, M., Lotze, M.T., 2010. Cancer and inflammation: promise for biologic therapy. J Immunother 33, 335-351.

Dmitriev, O.Y., 2011. Mechanism of tumor resistance to cisplatin mediated by the copper transporter ATP7B. Biochemistry and cell biology = Biochimie et biologie cellulaire 89, 138-147.

Fabbro, M., Henderson, B.R., 2003. Regulation of tumor suppressors by nuclear-cytoplasmic shuttling. Experimental cell research 282, 59-69.

Fink, D., Aebi, S., Howell, S.B., 1998. The role of DNA mismatch repair in drug resistance. Clinical cancer research : an official journal of the American Association for Cancer Research 4, 1-6.

Gazdar, A.F., Gao, B., Minna, J.D., 2010. Lung cancer cell lines: Useless artifacts or invaluable tools for medical science? Lung Cancer 68, 309-318.

Gelain, D.P., Dalmolin, R.J., Belau, V.L., Moreira, J.C., Klamt, F., Castro, M.A., 2009. A systematic review of human antioxidant genes. Frontiers in bioscience : a journal and virtual library 14, 4457-4463.

Gemma, A., Li, C., Sugiyama, Y., Matsuda, K., Seike, Y., Kosaihira, S., Minegishi, Y., Noro, R., Nara, M., Seike, M., Yoshimura, A., Shionoya, A., Kawakami, A., Ogawa, N., Uesaka, H., Kudoh, S., 2006. Anticancer drug clustering in lung cancer based on gene expression profiles and sensitivity database. BMC cancer 6, 174.

Howell, S.B., Safaei, R., Larson, C.A., Sailor, M.J., 2010. Copper transporters and the cellular pharmacology of the platinum-containing cancer drugs. Molecular pharmacology 77, 887-894.
Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., Forman, D., 2011. Global cancer statistics. CA: a cancer journal for clinicians 61, 69-90.
Kartalou, M., Essigmann, J.M., 2001. Recognition of cisplatin adducts by cellular proteins. Mutation research 478, 1-21.
Khan, N., Afaq, F., Mukhtar, H., 2010. Lifestyle as risk factor for cancer: Evidence from human studies. Cancer letters 293, 133-143.
Kinoshita, Y., Kalir, T., Rahaman, J., Dottino, P., Kohtz, D.S., 2012. Alterations in nuclear pore architecture allow cancer cell entry into or exit from drug-resistant dormancy. The American journal of pathology 180, 375-389.
Klamt, F., Zdanov, S., Levine, R.L., Pariser, A., Zhang, Y., Zhang, B., Yu, L.R., Veenstra, T.D., Shacter, E., 2009. Oxidant-induced apoptosis is mediated by oxidation of the actin-regulatory protein cofilin. Nature cell biology 11, 1241-1246.
Knudsen, N.O., Andersen, S.D., Lutzen, A., Nielsen, F.C., Rasmussen, L.J., 2009. Nuclear translocation contributes to regulation of DNA excision repair activities. DNA repair 8, 682-689.
Kuo, M.T., 2009. Redox regulation of multidrug resistance in cancer chemotherapy: molecular mechanisms and therapeutic opportunities. Antioxidants & redox signaling 11, 99-133.
Laborde, E., 2010. Glutathione transferases as mediators of signaling pathways involved in cell proliferation and cell death. Cell death and differentiation 17, 1373-1380.
Lonning, P.E., Knappskog, S., Staalesen, V., Chrisanthar, R., Lillehaug, J.R., 2007. Breast cancer prognostication and prediction in the postgenomic era. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 18, 1293-1306.
Martin, L.P., Hamilton, T.C., Schilder, R.J., 2008. Platinum resistance: the role of DNA repair pathways. Clinical cancer research : an official journal of the American Association for Cancer Research 14, 1291-1295.
Mountain, C.F., 1997. Revisions in the International System for Staging Lung Cancer. Chest 111, 1710-1717.
Muller, C.B., de Barros, R.L., Castro, M.A., Lopes, F.M., Meurer, R.T., Roehe, A., Mazzini, G., Ulbrich-Kulczynski, J.M., Dal-Pizzol, F., Fernandes, M.C., Moreira, J.C., Xavier, L.L., Klamt, F., 2011. Validation of cofilin-1 as a biomarker in non-small cell lung cancer: application of quantitative method in a retrospective cohort. Journal of cancer research and clinical oncology 137, 1309-1316.
Nair, A., Klusmann, M.J., Jogeesvaran, K.H., Grubnic, S., Green, S.J., Vlahos, I., 2011. Revisions to the TNM staging of non-small cell lung cancer: rationale, clinicoradiologic implications, and persistent limitations. Radiographics : a review publication of the Radiological Society of North America, Inc 31, 215-238.
Parker, R.J., Eastman, A., Bostick-Bruton, F., Reed, E., 1991. Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin-DNA lesions and reduced drug accumulation. The Journal of clinical investigation 87, 772-777.
Pedersen, M.O., Larsen, A., Stoltenberg, M., Penkowa, M., 2009. The role of metallothionein in oncogenesis and cancer prognosis. Progress in histochemistry and cytochemistry 44, 29-64.
Pendleton, A., Pope, B., Weeds, A., Koffer, A., 2003. Latrunculin B or ATP depletion induces cofilin-dependent translocation of actin into nuclei of mast cells. The Journal of biological chemistry 278, 14394-14400.
Perez, R.P., 1998. Cellular and molecular determinants of cisplatin resistance. Eur J Cancer 34, 1535-1542.

Pisters, K.M., Evans, W.K., Azzoli, C.G., Kris, M.G., Smith, C.A., Desch, C.E., Somerfield, M.R., Brouwers, M.C., Darling, G., Ellis, P.M., Gaspar, L.E., Pass, H.I., Spigel, D.R., Strawn, J.R., Ung, Y.C., Shepherd, F.A., 2007. Cancer Care Ontario and American Society of Clinical Oncology adjuvant chemotherapy and adjuvant radiation therapy for stages I-IIIA resectable non small-cell lung cancer guideline. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 25, 5506-5518.
Rabik, C.A., Dolan, M.E., 2007. Molecular mechanisms of resistance and toxicity associated with platinating agents. Cancer treatment reviews 33, 9-23.
Ramalingam, S., Belani, C., 2008. Systemic chemotherapy for advanced non-small cell lung cancer: recent advances and future directions. The oncologist 13 Suppl 1, 5-13.
Rosell, R., Lord, R.V., Taron, M., Reguart, N., 2002. DNA repair and cisplatin resistance in non-small-cell lung cancer. Lung Cancer 38, 217-227.
Siddik, Z.H., 2003. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 22, 7265-7279.
Siddik, Z.H., Mims, B., Lozano, G., Thai, G., 1998. Independent pathways of p53 induction by cisplatin and X-rays in a cisplatin-resistant ovarian tumor cell line. Cancer research 58, 698-703.
Sorokin, A.V., Kim, E.R., Ovchinnikov, L.P., 2007. Nucleocytoplasmic transport of proteins. Biochemistry (Moscow) 72, 1439-1457.
Sun, S., Schiller, J.H., Spinola, M., Minna, J.D., 2007. New molecularly targeted therapies for lung cancer. The Journal of clinical investigation 117, 2740-2750.
Teng, S.C., Wu, K.J., Tseng, S.F., Wong, C.W., Kao, L., 2006. Importin KPNA2, NBS1, DNA repair and tumorigenesis. Journal of molecular histology 37, 293-299.
Tew, K.D., 1994. Glutathione-associated enzymes in anticancer drug resistance. Cancer research 54, 4313-4320.
Torigoe, T., Izumi, H., Ishiguchi, H., Yoshida, Y., Tanabe, M., Yoshida, T., Igarashi, T., Niina, I., Wakasugi, T., Imaizumi, T., Momii, Y., Kuwano, M., Kohno, K., 2005. Cisplatin resistance and transcription factors. Current medicinal chemistry. Anti-cancer agents 5, 15-27.
van Staveren, W.C., Solis, D.Y., Hebrant, A., Detours, V., Dumont, J.E., Maenhaut, C., 2009. Human cancer cell lines: Experimental models for cancer cells in situ? For cancer stem cells? Biochimica et biophysica acta 1795, 92-103.
Voskoglou-Nomikos, T., Pater, J.L., Seymour, L., 2003. Clinical predictive value of the in vitro cell line, human xenograft, and mouse allograft preclinical cancer models. Clinical cancer research : an official journal of the American Association for Cancer Research 9, 4227-4239.
Wang, D., Lippard, S.J., 2005. Cellular processing of platinum anticancer drugs. Nature reviews. Drug discovery 4, 307-320.
Wang, W., Eddy, R., Condeelis, J., 2007. The cofilin pathway in breast cancer invasion and metastasis. Nature reviews. Cancer 7, 429-440.
Winton, T., Livingston, R., Johnson, D., Rigas, J., Johnston, M., Butts, C., Cormier, Y., Goss, G., Inculet, R., Vallieres, E., Fry, W., Bethune, D., Ayoub, J., Ding, K., Seymour, L., Graham, B., Tsao, M.S., Gandara, D., Kesler, K., Demmy, T., Shepherd, F., 2005. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. The New England journal of medicine 352, 2589-2597.
Zhu, C.Q., Ding, K., Strumpf, D., Weir, B.A., Meyerson, M., Pennell, N., Thomas, R.K., Naoki, K., Ladd-Acosta, C., Liu, N., Pintilie, M., Der, S., Seymour, L., Jurisica, I., Shepherd, F.A., Tsao, M.S., 2010. Prognostic and predictive gene signature for adjuvant chemotherapy in resected non-small-cell lung cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 28, 4417-4424.

Formatação da Revista Molecular Oncology

MOLECULAR ONCOLOGY

Molecular Oncology is published by Elsevier on behalf of the Federation of European Biochemical Societies

AUTHOR INFORMATION PACK

TABLE OF CONTENTS

ISSN: 1574-7891

DESCRIPTION

Molecular Oncology highlights new discoveries, approaches, as well as technical developments, in basic, clinical and discovery-driven translational research. Topics include:

• Key biological processes such as cell cycle; DNA repair; apoptosis; invasion and metastasis; angiogenesis and lymphangiogenesis; cell signalling and interactive networks; immune response.
• Emerging technologies (genomics, proteomics, functional genomics, metabolomics, tissue arrays, imaging), and model systems.
•Biomarkers: diagnosis, prognosis, stratification and efficacy.
• Cancer genetics, epigenetics, and genomic instability.
• Minimal residual disease, pre-malignant lesions.
• Cancer micro-environment.
• Molecular pathology.
• Tumour immunology.
• Translational research.
• Cancer therapy (target discovery, drug design, immunotherapy, combination therapies, resistance, and individualised treatment).
• Chemotherapy, radiotherapy and surgery.
• Clinical pharmacology.
• Clinical trials, integration of basic science into cancer clinical trials.
• Epidemiology and prevention.
• Infrastructures (biobanks, databases, genomic resources).

A main feature of the Journal is to provide an international forum for debating cancer issues, and for integrating the input of all the stakeholders.

Coverage: Reviews, original articles, technical notes, editorials, news & views (commentary, science policy issues, ethical and legal issues, patient organisations, industry needs and alliances, regulatory issues, news items), letters to the editor, conference announcements, advertisements.

Submitting Authors:
Manuscripts can be submitted to *Molecular Oncology* at: http://ees.elsevier.com/molonc/

AUDIENCE

Scientists and health professionals interested in molecular aspects of the development, diagnosis and treatment of cancer. Sponsored Articles: Molecular Oncology offers authors the option to sponsor non-subscriber access to their articles on Elsevier's electronic publishing platforms. For more information please view our http://www.elsevier.com/wps/find/authorsview.authors/sponsoredarticles Sponsored Article page .

IMPACT FACTOR

2010: 4.250 © Thomson Reuters Journal Citation Reports 2011

ABSTRACTING AND INDEXING

BIOSIS
EMBASE
EMBiology
Scopus

EDITORIAL BOARD

O. Kallioniemi, Turku, Finland
D.J. Kerr, Oxford, UK
G. Kroemer, Villejuif (Paris), France
O. C. Lingjaerde, Oslo, Norway
E. Liu, Singapore, Singapore
T.W. Mak, Toronto, ON, Canada
R. Marais, London, UK
E. Mardis, St. Louis, MO, USA
J. Massagué, New York, NY, USA
J. Mendelsohn, Houston, TX, USA
G.B. Mills, Houston, TX, USA
E. Oláh, Budapest, Hungary
M. Oren, Rehovot, Israel
M. Piccart, Brussels, Belgium
J.W. Pollard, New York, NY, USA
D.F. Ransohoff, Chapel Hill, NC, USA
J.S. Reis-Filho, London, UK
U. Ringborg, Stockholm, Sweden
H. Sasano, Sendai, Japan
A. Spatz, Montreal, QC, Canada
I.J. Stratford, Manchester, UK
F.C.G.J. Sweep, Nijmegen, Netherlands
D. Tarin, La Jolla, CA, USA
T. Tursz, Villejuif, France
M.L. Tykocinski, Philadelphia, PA, USA
M. Uhlen, Stockholm, Sweden
A. Ullrich, Singapore, Singapore
N. Urban, Seattle, WA, USA
J.E. Visvader, Parkville, VIC, Australia
A. Wellstein, Washington, DC, USA
O.D. Wiestler, Heidelberg, Germany
C.L. Willman, Albuquerque, NM, USA
H.-M. Yang, Beijing, China
Y. Yarden, Rehovot, Israel
J. Yates, La Jolla, CA, USA
T. Yeatman, Tampa, FL, USA
Q. Zhan, Beijing, China
T.F. Ørntoft, Aarhus N, Denmark

GUIDE FOR AUTHORS

INTRODUCTION

Molecular Oncology is an international comprehensive journal that publishes original research, editorial commentaries, review articles and news on molecular oncology. It highlights new discoveries, approaches, as well as technical developments, in basic, clinical and discovery-driven translational cancer research.

The emphasis is on work that significantly advances our understanding of disease processes leading to human tumour development and/or establishes novel concepts of clear clinical significance in diagnosis, prognosis and prevention strategies for cancer patients.

Topics include, but are not limited to:
Key biological processes such as cell cycle; DNA repair; apoptosis; invasion and metastasis; angiogenesis and lymphangiogenesis; cell signalling and interactive networks; immune response. Emerging technologies (genomics, proteomics, functional genomics, metabolomics, tissue arrays, bio-imaging), and model systems. Biomarkers: diagnosis, prognosis, stratification and efficacy. Cancer genetics, epigenetics, and genomic instability. Minimal residual disease, pre-malignant lesions. Cancer micro-environment. Molecular pathology. Tumour immunology. Translational research. Cancer therapy (target discovery, drug design, immunotherapy, combination therapies, resistance, and individualised treatment). Chemotherapy, radiotherapy and surgery. Clinical pharmacology. Clinical trials, integration of basic science into cancer clinical trials. Epidemiology. Infrastructures (biobanks, databases, genomic resources, technology platforms).

A main feature of the Journal is to provide an international Forum for debating cancer issues, and for integrating the input of all the stakeholders.

Types of paper
Full-length research articles, Technical Notes, News and Views, Reviews and Mini-Reviews, Letters to the Editor.

Contact details for submission
Papers should be submitted using the *Molecular Oncology* online submission system, http://ees.elsevier.com/molonc. For questions on the submission or reviewing process, please contact the Editorial Office prior to submission at e-mail: molonc@cancer.dk

Page charges
This journal has no page charges.

BEFORE YOU BEGIN

Ethics in publishing
For information on Ethics in publishing and Ethical guidelines for journal publication see http://www.elsevier.com/publishingethics and http://www.elsevier.com/ethicalguidelines.

Policy and ethics
The work described in your article must have been carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans* http://www.wma.net/en/30publications/10policies/b3/index.html; *EU Directive 2010/63/EU for animal experiments* http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; *Uniform Requirements for manuscripts submitted to Biomedical journals* http://www.icmje.org. This must be stated at an appropriate point in the article.

Conflict of interest
All authors are requested to disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. See also http://www.elsevier.com/conflictsofinterest.

Submission declaration
Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or

explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere including electronically in the same form, in English or in any other language, without the written consent of the copyright-holder.

Authorship

All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:
Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed.
After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (for more information on this and copyright see http://www.elsevier.com/copyright). Acceptance of the agreement will ensure the widest possible dissemination of information. An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.
Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations (please consult http://www.elsevier.com/permissions). If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: please consult http://www.elsevier.com/permissions.

Retained author rights

As an author you (or your employer or institution) retain certain rights; for details you are referred to: http://www.elsevier.com/authorsrights.

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated. Please see http://www.elsevier.com/funding.

Funding body agreements and policies
Elsevier journals comply with current NIH public access policy

Open access

This journal offers you the option of making your article freely available to all via the ScienceDirect platform. To prevent any conflict of interest, you can only make this choice after receiving notification that your article has been accepted for publication. The fee of $3,000 excludes taxes and other potential author fees such as color charges. In some cases, institutions and funding bodies have entered into agreement with Elsevier to meet these fees on behalf of their authors. Details of these agreements are available at http://www.elsevier.com/fundingbodies. Authors of accepted articles, who wish to take advantage of this option, should complete and submit the order form (available at

http://www.elsevier.com/locate/openaccessform.pdf). Whatever access option you choose, you retain many rights as an author, including the right to post a revised personal version of your article on your own website. More information can be found here: http://www.elsevier.com/authorsrights .

Language and language services

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who require information about language editing and copyediting services pre- and post-submission please visit http://webshop.elsevier.com/languageservices or our customer support site at http://support.elsevier.com for more information.

Submission

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts source files to a single PDF file of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF files at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail removing the need for a paper trail.

Reviewers

Please submit, with the manuscript, the names, addresses and e-mail addresses of 3 potential reviewers. Note that the editor retains the sole right to decide whether or not the suggested reviewers are used.

PREPARATION

Use of wordprocessing software

It is important that the file be saved in the native format of the wordprocessor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the wordprocessor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: http://www.elsevier.com/guidepublication). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.
To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your wordprocessor.

Article structure

Subdivision - numbered sections

Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions
The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices
If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

- ***Title.*** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- ***Author names and affiliations.*** Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.
- ***Corresponding author.*** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address. Contact details must be kept up to date by the corresponding author.**
- ***Present/permanent address.*** If an author has moved since the work described in the article was done, or was visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

A Graphical abstract is optional and should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership online. Authors must provide images that clearly represent the work described in the article. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. See http://www.elsevier.com/graphicalabstracts for examples.
Authors can make use of Elsevier's Illustration and Enhancement service to ensure the best presentation of their images also in accordance with all technical requirements: Illustration Service.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). See http://www.elsevier.com/highlights for examples.

Keywords

Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Database linking

Elsevier aims at connecting online articles with external databases which are useful in their respective research communities. If your article contains relevant unique identifiers or accession numbers (bioinformatics) linking to information on entities (genes, proteins, diseases, etc.) or structures deposited in public databases, then please indicate those entities according to the standard explained below.

Authors should explicitly mention the *database abbreviation (as mentioned below) together with the actual database number*, bearing in mind that an error in a letter or number can result in a dead link in the online version of the article.

Please use the following format: **Database ID: xxxx**

Links can be provided in your online article to the following databases (examples of citations are given in parentheses):

- ASTM: ASTM Standards Database (ASTM ID: G63)
- CCDC: Cambridge Crystallographic Data Centre (CCDC ID: AI631510)
- GenBank: Genetic sequence database at the National Center for Biotechnology Information (NCBI) (GenBank ID: BA123456)
- GEO: Gene Expression Omnibus (GEO ID: GSE27196; GEO ID: GPL5366; GEO ID: GSM9853)
- MI: EMBL-EBI OLS Molecular Interaction Ontology (MI ID: 0218)
- MINT: Molecular INTeractions database (MINT ID: 6166710)
- NCBI Taxonomy: NCBI Taxonomy Browser (NCBI Taxonomy ID: 48184)
- NCT: ClinicalTrials.gov (NCT ID: NCT00222573)
- OMIM: Online Mendelian Inheritance in Man (OMIM ID: 601240)
- PDB: Worldwide Protein Data Bank (PDB ID: 1TUP)
- TAIR: The Arabidopsis Information Resource database (TAIR ID: AT1G01020)
- UniProt: Universal Protein Resource Knowledgebase (UniProt ID: Q9H0H5)

Math formulae

Present simple formulae in the line of normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article, using superscript Arabic numbers. Many wordprocessors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article. Do not include footnotes in the Reference list.

Table footnotes

Indicate each footnote in a table with a superscript lowercase letter.

Artwork

Electronic artwork

General points

- Make sure you use uniform lettering and sizing of your original artwork.
- Save text in illustrations as 'graphics' or enclose the font.
- Only use the following fonts in your illustrations: Arial, Courier, Times, Symbol.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Provide captions to illustrations separately.
- Produce images near to the desired size of the printed version.
- Submit each figure as a separate file.

A detailed guide on electronic artwork is available on our website:
http://www.elsevier.com/artworkinstructions
You are urged to visit this site; some excerpts from the detailed information are given here.

Formats
Regardless of the application used, when your electronic artwork is finalised, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):
EPS: Vector drawings. Embed the font or save the text as 'graphics'.
TIFF: Color or grayscale photographs (halftones): always use a minimum of 300 dpi.
TIFF: Bitmapped line drawings: use a minimum of 1000 dpi.
TIFF: Combinations bitmapped line/half-tone (color or grayscale): a minimum of 500 dpi is required.
If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is'.
Please do not:
- Supply files that are optimised for screen use (e.g., GIF, BMP, PICT, WPG); the resolution is too low;
- Supply files that are too low in resolution;
- Submit graphics that are disproportionately large for the content.

Color artwork
Please make sure that artwork files are in an acceptable format (TIFF, EPS or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color on the Web (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article.** Please indicate your preference for color: in print or on the Web only. For further information on the preparation of electronic artwork, please see http://www.elsevier.com/artworkinstructions.
Please note: Because of technical complications which can arise by converting color figures to 'gray scale' (for the printed version should you not opt for color in print) please submit in addition usable black and white versions of all the color illustrations.

Figure captions
Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables
Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

References
Citation in text
Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Web references
As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue
Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference management software
This journal has standard templates available in key reference management packages EndNote (http://www.endnote.com/support/enstyles.asp) and Reference Manager (http://refman.com/support/rmstyles.asp). Using plug-ins to wordprocessing packages, authors only need to select the appropriate journal template when preparing their article and the list of references and citations to these will be formatted according to the journal style which is described below.

Reference style
Text: All citations in the text should refer to:
1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;
2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.
Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.
Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ....'
List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.
Examples:
Reference to a journal publication:
Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.
Reference to a book:
Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.
Reference to a chapter in an edited book:
Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Journal abbreviations source
Journal names should be abbreviated according to
Index Medicus journal abbreviations: http://www.nlm.nih.gov/tsd/serials/lji.html;
List of title word abbreviations: http://www.issn.org/2-22661-LTWA-online.php;
CAS (Chemical Abstracts Service): http://www.cas.org/sent.html.

Video data
Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. In order to ensure that your video or animation material is directly usable, please provide the files in one of our recommended file formats with a preferred maximum size of 50 MB. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages at http://www.elsevier.com/artworkinstructions. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Supplementary data
Elsevier accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please provide the data in one of our recommended file formats. Authors should

submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/artworkinstructions.

Submission checklist

The following list will be useful during the final checking of an article prior to sending it to the journal for review. Please consult this Guide for Authors for further details of any item.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address
- Telephone and fax numbers

All necessary files have been uploaded, and contain:
- Keywords
- All figure captions
- All tables (including title, description, footnotes)

Further considerations
- Manuscript has been 'spell-checked' and 'grammar-checked'
- References are in the correct format for this journal
- All references mentioned in the Reference list are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Web)
- Color figures are clearly marked as being intended for color reproduction on the Web (free of charge) and in print, or to be reproduced in color on the Web (free of charge) and in black-and-white in print
- If only color on the Web is required, black-and-white versions of the figures are also supplied for printing purposes

For any further information please visit our customer support site at http://support.elsevier.com.

Editorial timeline

The editors will make every effort to reach decisions on submitted papers within 4 weeks of the submission date. If revisions are a condition of publication, we generally allow two months for revisions and consider only one revised version of the paper. Evaluations of conceptual advance and significance are made based on the literature available on the day of the final decision, not the day of submission. Any major changes after acceptance are subject to review and may delay publication.

AFTER ACCEPTANCE

Use of the Digital Object Identifier

The Digital Object Identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alpha-numeric character string which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. The correct format for citing a DOI is shown as follows (example taken from a document in the journal *Physics Letters B*):

doi:10.1016/j.physletb.2010.09.059

When you use the DOI to create URL hyperlinks to documents on the web, the DOIs are guaranteed never to change.

Proofs

One set of page proofs (as PDF files) will be sent by e-mail to the corresponding author (if we do not have an e-mail address then paper proofs will be sent by post) or, a link will be provided in the e-mail so that authors can download the files themselves. Elsevier now provides authors with PDF proofs which can be annotated; for this you will need to download Adobe Reader version 7 (or higher) available free from http://get.adobe.com/reader. Instructions on how to annotate PDF files will accompany the proofs (also given online). The exact system requirements are given at the Adobe site: http://www.adobe.com/products/reader/tech-specs.html.

If you do not wish to use the PDF annotations function, you may list the corrections (including replies to the Query Form) and return them to Elsevier in an e-mail. Please list your corrections quoting line number. If, for any reason, this is not possible, then mark the corrections and any other comments (including replies to the Query Form) on a printout of your proof and return by fax, or scan the pages and e-mail, or by post. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. We will

do everything possible to get your article published quickly and accurately – please let us have all your corrections within 48 hours. It is important to ensure that all corrections are sent back to us in one communication: please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility. Note that Elsevier may proceed with the publication of your article if no response is received.

Offprints

The corresponding author, at no cost, will be provided with a PDF file of the article via e-mail. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. The PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use.

Distribution of materials and data

One of the terms and conditions of publishing in *Molecular Oncology* is that authors be willing to distribute any materials and protocols used in the published experiments to qualified researchers for their own use. Materials include, but are not limited to, cells, DNA, antibodies, reagents, organisms, and mouse strains or, if necessary, the relevant ES cells. These must be made available with minimal restrictions and in a timely manner, but it is acceptable to request reasonable payment to cover the cost of maintenance and transport of materials. If there are restrictions to the availability of any materials, data, or information, these must be disclosed in the cover letter and the experimental procedures section of the manuscript at the time of submission. Nucleic acid and protein sequences, macromolecular structures determined by X-ray crystallography (along with structure factors), and microarray data must be deposited in the appropriate public database and must be accessible without restriction from the date of publication. An entry name or accession number must be included at the last paragraph of the Experimental Procedures section in the final version of the manuscript. Microarray data should be MIAME compliant (for guidelines see http://www.mged.org/Workgroups/MIAME/miame.html).

In addition to the information that must be deposited in public databases as detailed above, authors are encouraged to contribute additional information to the appropriate databases. Authors are also encouraged to deposit materials used in their studies to the appropriate repositories for distribution to researchers.

Open Archive

Molecular Oncology papers are freely available starting 12 months after publication.

AUTHOR INQUIRIES

For inquiries relating to the submission of articles (including electronic submission) please visit this journal's homepage. Contact details for questions arising after acceptance of an article, especially those relating to proofs, will be provided by the publisher. You can track accepted articles at http://www.elsevier.com/trackarticle. You can also check our Author FAQs (http://www.elsevier.com/authorFAQ) and/or contact Customer Support via http://support.elsevier.com.