Expert Review of Molecular Diagnostics

ISSN: 1473-7159 (Print) 1744-8352 (Online) Journal homepage: informahealthcare.com/journals/iero20

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To cite this article: Dimitrios H Roukos, Christos Katsios & Theodore Liakakos (2010) Genotype–phenotype map and molecular networks: a promising solution in overcoming colorectal cancer resistance to targeted treatment, Expert Review of Molecular Diagnostics, 10:5, 541-545, DOI: 10.1586/erm.10.49

To link to this article: https://doi.org/10.1586/erm.10.49

Genotype–phenotype map and molecular networks: a promising solution in overcoming colorectal cancer resistance to targeted treatment

Expert Rev. Mol. Diagn. 10(5), 541–545 (2010)

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"The goal is to link genomics data with clinical data in order to understand why some patients respond to therapy and are cured, while others experience fatal metastatic recurrence."

Despite traditional molecular research advances being translated into approved targeted agents, including cetuximab, panitumumab and bevacizumab, the overall survival benefit of patients with colorectal cancer (CRC) is small. At the end of the first postgenomic decade, emerging genomics data revealed a high complexity and heterogeneity of the disease, which explains the clinical limitations of classic single-gene research. Here we discuss whether and how 'big' biology and science for completing the cancer mutations catalog and advances in systems biology and molecular networks modeling may lead to a genotype–phenotype map. This relationship prediction can lead to the next-generation of biomarkers and biologic agents to change poor outcomes of advanced CRC.

Genetic alterations & signaling pathways deregulation

Nearly two decades ago, mutations in a Wnt-pathway component, the gene adenomatous polyposis coli (*APC*), were described to activate signaling and were associated with the majority of colon cancers [1]. The duration of this tumorigenic process, from small benign tumors (adenomas) to invasive adenocarcinomas, is long, and often several years or even decades are needed for diagnostic evidence. Thus, given this long-term evolution, it is recommended that adults older than 50 years are screened, by a minimal invasive approach, in order to identify and resect adenomas, polyps or early-stage CRC, as a safe and effective prevention of the disease [101].

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Cell proliferation, growth, survival and apoptosis is regulated by signals that are transmitted from cell surface receptors to its nucleus through various signaling pathways. When mutations occur and accelerate in key genes (components of downstream pathways), they deregulate the signal transduction and the aforementioned cellular processes, including angiogenesis, causing cancer development. Therefore, inhibition of these pathways through targeted agents represents an attractive anticancer therapy,

Indeed, the concept of biologic agents has been incorporated into the pharmaceutical industry over the last decade. Targeting only cancer cells and not healthy cells when using biologics could dramatically improve the poor outcomes of cancer patients, while maintaining very low adverse effect profiles. These druggable targets have aroused excitement in the biotechnology industry for drug discovery, including two major categories of drugs: monoclonal antibodies and small-molecule tyrosine kinase inhibitors (TKIs). The mechanisms of action of these two agent

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 10.1586/ERM.10.49 ISSN 1473-7159

categories differ. For example, the monoclonal antibody trastuzumab (for the treatment of HER2-positive breast cancer) binds to the extracellular portion of HER2, blocks the ligand-receptor dimerization and, thus, inhibits signal transduction from outside the cell to the nucleus. By contrast, the EGFR TKIs erlotinib and gefitinib (developed for the treatment of non-small-cell lung cancer and other tumors) compete reversibly with ATP to bind to the intracellular domain of EGFR, thus inhibiting EGFR autophosphorylation and downstream signaling [2]. However, despite these major molecular research advances, for most solid tumors, the overall clinical success of biologically targeted agents is limited. Positive results from Phase III randomized controlled trials have been reported for trastuzumab treatment in HER2-positive breast cancer and gastric cancer [3,4]. However, such evidence from comparative–effectiveness research, which is currently considered essential for wide clinical use of new agents [5], has not yet emerged for several other major cancer types [6–8].

Targeting colorectal cancer

Distinct signaling pathways, including Wnt, EGFR and VEGF have a crucial role in tumor initiation and progression. The Wnt signaling pathway is fascinating for three main reasons: first, it is more often activated in CRC than other pathways; second, because of the biological functions it controls; and third, because of its unusual mechanism for transmitting information to the cell nucleus. Most pathways transmit information through a chain of enzymes, known as kinases, which work by transferring phosphate groups to molecules, and which are relatively easy to inhibit. By contrast, the Wnt pathway transmits signals by controlling the relative stabilities of two proteins. The first of these is axin, a scaffold protein on which is built a 'destruction complex' that destabilizes the second protein, β-catenin [9]. A recent study revealing a compound, known as XAV939, that potently inhibits Wnt signaling could change our fundamental understanding of Wnt signaling, leading to drugs that target Wnt-dependent cancers [10].

> "...despite these major molecular research advances, for most solid tumors, the overall clinical success of biologically targeted agents is limited."

In contrast to Wnt signaling, several anti-EGFR (cetuximab, panitumumab), and anti-VEGF (bevacizumab) agents have been developed and have demonstrated some efficacy in the treatment of metastatic CRC.

Clinical trials

Efficacy & resistance

Systemic chemotherapy with a fluorouracil-based treatment combined with either irinotecan or oxaliplatin is the standard of care in CRC treatment. In the metastatic setting, chemotherapy has improved overall survival to more than 20 months [11]. In the adjuvant setting, it provides a clear overall survival benefit. Indeed, a recent study by the Adjuvant Colon Cancer Endpoints Group analyzed the dataset from 18 trials and more than 20,800 Stage II or III colon cancer patients testing fluorouracil-based adjuvant therapy. At a median follow-up period of 8 years, this chemotherapy significantly reduced the risk of recurrence after complete surgical resection (R0) to 35%. Given that recurrence events rarely occur after 8 years or more following treatment, the authors point out the importance of adjuvant chemotherapy to improve cure rates [12]. How could overall survival be further improved?

Antibodies

Five randomized controlled trials testing the safety and efficacy of the addition of cetuximab or panitumumab to chemotherapy alone or plus bevacizumab in metastatic CRC have been published [13–17], Table 1 summarizes the results of these studies, which included 3896 patients. The addition of these anti-EGFR antibodies significantly improved progression-free survival only for *KRAS* wild-type disease. As a result, recent guideline recommendations suggest consideration of these antibodies. Therefore, oncologists now increasingly use these agents in patients with metastatic CRC. However, more recently, valid concerns have emerged that limit the initial excitement.

Limitations

In order to balance the risks and benefits, and despite approval, more rigorous criteria are required for the wide clinical use of cetuximab or panitumab in addition to chemotherapy alone or with bevacizumab in metastatic CRC. The absence of any overall survival benefit, with a progression-free survival benefit only among *KRAS* wild-type tumors, as well as the adverse effects of these drugs raises several questions. First, the use of progression-free survival in an absence of overall survival gain is now increasingly questionable. Intratumoral heterogeneity suggests that although most cancer cells are sensitive to these agents, resistant small cancer cell subpopulations are rapidly proliferated, causing tumor re-growth and new metastases [18,19]. Second, even progression-free survival improvement does not meet rigorous evidence assessment, because, in fact, it was a retrospective analysis of KRAS status in tumor specimens when no therapeutic effect of these agents was found in the overall patient population. Third, although limited, cetuximab or panitumumab use increases the risk of adverse events, including skin reactions, infusion-related reactions and diarrhea [13–17].

Adjuvant setting

Can biologic agents reduce risk of recurrence in stage II and III colorectal cancer improving disease-free survival and cure rates? The absence of overall survival benefit in the metastatic setting is suggestive of the inability of the treatment to eliminate all cancer cells, and reduces the expectations for clinical success in the adjuvant setting. However, the limited tumor burden with presence only of micrometastatic disease in stage II and III might be eliminated by the therapeutic effect of the biologic agents, Therefore, before the results of near-complete or ongoing trials (bevacizumab: NSABP C-08, AVANT, E5202, Quick and Simple and Reliable Collaborative Group-2, NCCTG, N0147; cetuximab: PETACC-8) become available, no definitive

Table 1. Phase III randomized controlled trials testing the efficacy and safety of cetuximab or panitumab added to chemotherapy alone or plus bevacizumab in metastatic colorectal cancer.

All patients				*KRAS* status				Ref.
Study	*Patients (n)*	*Chemotherapy ± cetuximab or panitumumab*	*OS*	*PFS (HR, 95% [CI])*		*OS (HR, 95% [CI])*		
				Wild-type KRAS	*Mutant* KRAS	*Wild-type* KRAS	*Mutant* KRAS	
CRYSTAL trial	1198	599 vs 599	NS	0.68 (0.50–0.94) (p = 0.02)	1.07 (0.71–1.61) (p = 0.75)	0.84 (0.64–1.11)	1.03 (0.74–1.44)	[13]
Tol CAIRO2 trial	736	368 vs 368	NS	No HR (p = 0.3)	No HR (p = 0.003)	No HR (p = 0.64)	No HR (p = 0.03)	[14]
Karapetis AGITG CO.17 trial	572	285 vs 287	NS	0.40 (0.30–0.54) (p < 0.001)	0.99 (0.73–1.35) (p = 0.96)	0.55 (0.41–0.74) (P<0.001)	0.98 (0.70–1.37) (p = 0.89)	[15]
Hecht[1]	823	413 vs410	NS	1.36 (1.04–1.77)	1.25 (0.91–1.71)	1.89 (1.30–2.75)	1.02 (0.67–1.54)	[16]
Hecht[2]	230	115 vs 115	NS	1.5 (0.82–2.76)	1.19 (0.65–2.21)	1.28 (0.50–3.25)	2.14 (0.82–5.59)	[16]
Bokemeyer OPUS trial	337	169 vs 168	NA	0.57 (0.358–0.907) (p = 0.016)	1.83 (1.095–3.056) (p = 0.019)	NA	NA	[17]

AGITG CO.17 Trial: Cetuximab vs best supportive care; CAIRO2 trial: Capecitabine + oxaliplatin+ bevacizumab ± cetuximab; CRYSTAL trial: FOLFIR ± cetuximab; Hecht[1]: FOLFOX -4 + bevacizumab ± panitumumab; Hecht[2]: FOLFIR + bevacizumab ± panitumumab; OPUS trial: FOLFOX -4 ± cetuximab.
5-FU: Fluorouracil; Ab: Antibody; AGITG CO.17 Trial: Australasian Gastro-Intestinal Trials Group; CAIRO2 trial: Capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer; CRYSTAL trial: Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer; FOLFIR: Folinic acid (leucovorin), 5-FU, irinotecan (Campostar); FOLFOX: Folinic acid (leucovorin), 5-FU, oxaliplatin; HR: Hazard ratio; NA: Not available; NE: Not estimated; OPUS trial: Oxaliplatin and Cetuximab in the First-Line Treatment of Metastatic Colorectal Cancer; OS: Overall survival; PFS: Progression-free survival.

conclusions can be drawn regarding whether these agents have a recurrence-delaying, curative or no effect in the adjuvant treatment of CRC [12].

A decade after the first two completed drafts of the human genome sequence, both our understanding of genetic and molecular basis of cancer, and insights into molecular mechanisms underlying tumor development and metastasis have been changed dramatically [20,21].

DNA sequencing technology explosion

The first postgenome decade was characterized by spectacular advances in genome science [22]. As the costs dramatically drop and the sequencing data quality is improved, dozens of complete sequence human genomes have been published and there are close to 200 unpublished ones [20]. At least three fully sequenced cancer genomes, including breast, lung and melanoma cancer have been published [23].

> "...this genomic revolution has not yet translated into cancer genomics-based oncology."

'Big biology' efforts, such as the International HapMap Project, and the Encyclopedia of DNA Elements (ENCODE), provide important information on gene-coding and noncoding DNA, aiming to improve our understanding in every functional element in the human genome. Much noncoding DNA has a regulatory role; small RNAs of different varieties seem to control gene expression at the level of both DNA and RNA transcripts in ways that are only beginning to become clear [21,22]. However, this genomic revolution has not yet translated into cancer genomics-based oncology.

Cancer complexity

The gap between basic research and clinical application has widened. The more we learn, the bigger the problem of understanding life diversity and complex disease pathogenesis and evolution, such as cancer [21,23]. The implication of a genomic revolution into medicine and oncology are limited. To move forward to the future, Collins considers five key lessons: personalized medicine, technology, policy, partnerships and pharmacogenomics [24]. Craig Venter emphasizes the need for research on linking genotype to phenotype, and points out that because of myriad phenotypic traits, more powerful computational strategies will be needed to link phenotype to genotype [25].

Current drugs generation: modest expectations

How could we explain the limitations of the current generation of biologic agents in the treatment of solid cancers, including CRC? Emerging evidence reveals that the landscape of mutations and deregulated signaling pathways underlying cancer development and progression of solid cancers is much more complex than we could imagine. First, recent studies using massively parallel DNA sequencing technology have revealed, that not only point mutations (e.g., nucleotide insertions, deletions and SNPs), but also genomic rearrangements and copy-number changes are involved in tumorigenesis of major cancers [26–28]. Although the costs for full-genome sequencing may drop to approximately US$1000 in the next few years, allowing thousands of cancer genomes to be sequenced, major challenges cause uncertainty. The discrimination between causal (driver) and noncausal (passenger) mutations still remains a challenge. But the next big challenge is to explore and understand the functional role of mutations in the nonprotein-coding genome [21,23].

The second and even greater problem is how to understand the complex gene–gene, protein–protein, gene–environment and cancer cell–cell interactions in a timely dynamic process. It is thought that the inference of an oncological outcome, namely tumorigenesis or metastatic recurrence, is driven by complex molecular networks rather than a simple linear relationship between genetic alterations and phenotype [29–33].

Genotype–phenotype map

Complete data for both genotype and phenotype are crucial in the effort to predict survival and risk of recurrence. Several studies have already identified a large number of mutations and genes involved in CRC [28,34]. With cheaper and more reliable sequencing technology, it is expected that ful and partial CRC genome sequences will improve the catalog of driver mutations, including point mutation rearrangements and copy-number changes. From a phenotype perspective, high-quality clinical, pathological, therapeutic and follow-up data (phenotype) are available from large-scale randomized controlled trials and databases. Although still in its infancy, efforts are underway to link a phenotypic event (recurrence, death) with genetic alterations (genotype).

> "Emerging evidence reveals that the landscape of mutations and deregulated signaling pathways underlying cancer development and progression of solid cancers is much more complex than we could imagine."

The understanding of a nonlinear relationship between genotype and phenotype is a major problem that might be overcome with the evolution of both biomedical and mathematical sciences. Several computational strategies are being developed to predict gene–gene and gene–environment interactions [31–33]. Bionetwork modeling represents one of the most fascinating fields towards a genotype–phenotype-based personalized medicine [35]. Efforts are underway to integrate genotyping and molecular data into molecular network modeling to predict outcomes [32]. The systems biology approach shapes a new way to understand complex biological systems, such as individual tumor, host and environment. The goal is to link genomics data with clinical data in order to understand why some patients respond to therapy and are cured, while others experience fatal metastatic recurrence. Given that the mutations catalog has to be completed, bionetworks modeling with emphasis on completed clinical data available might accelerate the development of the next generation of network-based biomarkers and biologically targeted agents [36].

Beyond EGFR and VEGF, other signaling pathways (e.g., Wnt) drive the oncological outcome. Given the interconnections of the signaling pathway components, and the heterogeneity of the deregulated pathways among CRC individual patients, it is important not only to characterize which pathways are activated in a certain patient, but also the inference of these interactions. A systems biology approach to the prediction of signal transduction that includes input (receptors), intracellular signaling pathways network and output (nucleus) is crucial for understanding intratumoral single cancer cell function and their interactions.

Conclusion

Adequate surgery with complete tumor resection and adjuvant chemotherapy have improved survival and cure rates of patients with non-metastatic CRC. In the metastatic setting, modern chemotherapy significantly prolongs survival. The present generation of targeted agents has initially provided excitement, but results from ongoing and new randomized controlled trials should be awaited for definitive conclusions about their efficacy.

At the end of the first postgenomic decade with an explosion in sequencing technology, important advances and insights into genetic variation and molecular mechanisms underlying human diversity have improved our understanding of cancer complexity and heterogeneity. This high complexity of cancer, revealed at an unprecedented level using parallel sequencing technology, explains the small impact, at least to date, of genomics and current biologic agents in the day-to-day clinical practice of CRC.

Bionetworks modeling and systems biology approaches provide a fascinating field for the development of the next generation of biomarkers and biologic agents. Advances with the next-generation DNA sequencing technology, along with conceptual innovation, may overcome the current myriad challenges in genotype–phenotype-based personalized cancer medicine.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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