Inter-tumor heterogeneity

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Advances in the molecular study of cancer have focused on biomarkers in the setting of tumor-driving mutations within the great heterogeneity of the tumor genomic landscape. It is clearly recognized now that even two tumors originating from the same organ even if histological they appear similar their behavior and response to therapy can be different. These findings have increased interest and research to find truly prognostic and predictive biomarkers to serve as tools in better assessing the natural course of disease and response to treatments in the hope of truly individualizing cancer therapy in the future.

Even as pathologists started to recognize the origin of different tumors by histology and with standard immuno-histochemistry, they postulated the tissue of origin of metastatic lesions; the reason why some tumors behaved in a more aggressive way than others from the same origin, or why some tumors would benefit from certain treatments while others would actually have a worse prognosis with treatment was poorly understood.

A good example of how practice patterns have changed based on the way that we have been increasing our knowledge of the biology of the tumors is with Colon Cancer. For many years we felt that the only important feature of tumors was their degree of differentiation, with poorly differentiated tumors originating from more primitive cells and hence having a more aggressive cell growth.

Subsequently, the recognition of different enzymatic levels within the tumors was felt to account for the response to fluoropyrimidines, as it was the case with the discovery of thymidylate synthase. Tumors that have low expression of this enzyme have an improved response to treatment with fluoropyrimidines, but that effect is lost if therapy is combined with other chemotherapeutic agents such as irinotecan.[1] More recently, with the genetic studies of tumors from colonic origin it was clearly recognized that microsatellite instability was an important biomarker that could explain those patterns of behavior and the recognition that tumors of the right colon were more prone to mismatch repair deficiency opened the door to a better understanding of the behavior of this tumor in its early stages.

Much to our surprise, mismatch repair deficient cancers, also known as microsatellite instability high (suggest high-frequency microsatellite instability), that tend to have a better prognosis in early stages were found to have a detrimental effect of adjuvant therapy with 5-Fluorouracil.[2] This fact started to change the way we manage at least stage II colon cancer. This was quite likely the first recognition of inter-tumor heterogeneity that was clearly relevant to clinicians in practice. However, there are still reports that close to 50% of oncologists would still administer chemotherapy to this patient population. This is a clear example of how clinicians have been slow in adopting some genetic and molecular biomarkers in Colon cancer, which has prompted prospective clinical studies that are trying to answer these questions. One such study is the Eastern cooperative group study 5202, in which high risk patients defined as microsatellite instability high (high-frequency microsatellite instability) or patients with loss of heterozygocity at chromosome 18q were assigned to receive adjuvant therapy while low risk patients were only observed.

Then as we began to offer patients treatments with targeted agents, regulatory agencies were fast to try to "select" which patients would benefit from the therapy. However, given the poor understanding we had at the time of the prognostic and predictive markers, the initial approval was based mostly on expression of the target. This happened with the advent of epidermal growth factor inhibitors (EGFR) which were initially only approved for patients with high expression of the EGFR receptor, even though this was never proven in a prospective fashion to be predictive of response to these agents. More recently different signaling pathways have been described; with special interest in the ones that pertain to cell survival and division, but in colon cancer the understanding of the KRAS pathway has been quite likely the most important advance toward a personalized medicine. Showing not only an independent prognostic importance[3] but also a predictive behavior in which patients with a mutation in the KRAS did not experience any benefit from treatment with EGFR.[4]

Even as we started to understand that pathway, and KRAS being the first step in the activation of the trans-membrane receptor of EGFR, it took several years to show that if we only excluded the patients with the KRAS mutation from receiving treatment with these agents, which constitutes approximately 40% of the patients with colon cancer, an additional 20–30% of the remaining patients would still have no benefit from treatment with EGFR inhibition. This meant that some other markers were not "yet" integrated in the treatment decisions, and as we started to add the downstream markers in the pathway a better patient selection became possible.[5] These findings have also become apparent as the data from prior research involving these agents was retrospectively studied. Significance was found for BRAF and NRAS mutations; however this last one occurs in only 3–5% of patients. In order to avoid exposing patients to toxic agents unnecessarily it is worthwhile to know the molecular behavior of each gene in each patient.

When studying a biomarker one must carefully decide if the interest is to find if the specific marker will be prognostic (what will be the patient outcome regardless of therapy), predictive (how can we affect the outcome with specific/targeted therapies) or both. Thus far, the arena in colon cancer is not crowded with those to say the least. While MSI, KRAS and BRAF have proven to be both predictive and prognostic, NRAS has shown only prognostic significance. But many other potential biomarkers are being investigated, mostly on the KRAS pathway and it is quite likely that better patient selection will be able to be applied when deciding who to expose to specific therapies. At this time we expose the totality of the KRAS wild type population to EGFR inhibitors, which compromises 60% of all colon cancer despite knowing that only 30% will benefit from this therapy. If we start subtracting all other known biomarkers such as BRAF mutant (8–10%), PTEN null (10–15%) and PI3KC mutant (10–12%) we might be able to expose only the 30% that will actually benefit from such a therapy, with the economical and toxicology implications that were mentioned previously.

Another EGFR pathway in which, not a mutation but a loss of function, would confer resistance to the treatment is the PTEN pathway which culminates with hyperphosphorylation of AKT protecting cancer cells from apoptotic stimuli and more importantly separating two different tumor types.

The KRAS and the PTEN pathways are independent prognostic factors in colon cancer, but it is more important to understand if these molecular changes are mutually exclusive to the point that we could separate the tumors and decide on treatment based on an organized testing. This would avoid having to test each tumor for all possible mutations, which would be both expensive and make it difficult to interpret.[6] At this time there is increased interest in designing trials that will prospectively evaluate the EGFR pathway markers, receptor polymorphism and ligands not only for targeted agents but also for cytotoxic agents that are currently incorporated in the treatment of colon cancer.

Recently we have expanded our therapeutic options to Vascular Endothelial Growth Factor (VEGF) receptor antagonists, but it has proven more difficult to validate inter-tumor heterogeneity in the stroma of these cancers since VEGF plays a bigger role in the support system that the tumor builds as it grows, so it might not show the same inter-tumor heterogeneity. We have yet to identify biomarkers that would help us select which patients would benefit from this therapy. One of the problems here could be that we are devoting our interest mainly on the cancer cell itself and not enough on the surrounding tissues, which in the end is the target of these medications.

Cell signaling pathways and their interaction is the main focus of multiple studies, since once a pathway is blocked, the mechanism of resistance could be elicited by interlinked pathways involved in cell proliferation and ultimately in progression of the cancer.[7]

Even as we understand the most specific mutation in the cell metabolism and we target it with a very specific agent, there is still the possibility that other patient related factors could influence in the response to different agents, as it has been recently reported that other factors such as baseline quality of life, baseline platelet count (likely a factor of inflammation), and appearance of skin rash could be predictors of longer survival and response to treatment with targeted agents that one would consider to have little to do with those factors.

Another interesting field of research is to evaluate how the tumors are resistant to therapies and how can we alter that mechanism of resistance in an effort to be able to offer the patients all available therapies; with up to eight mechanisms of resistance including decrease ubiquitylation of the EGFR, modulation of the EGFR by Src family kinases and other mutations in the downstream pathway. There are even reports of the mechanism of resistance being the overexpression of the vascular endothelial growth factor (VEGF) leading to escape from EGFR therapy.

The importance of biomarkers in this era has become apparent for many reasons; in the past oncology was spared from the critical dogma "first do no harm" and we could give toxic treatments to many patients who would only be receiving the side effects with no benefit whatsoever,

and even in some cases a detrimental effect of the treatments with a worsening survival on the treatment arms. Also as the technology advances and the cost of medications increases, the need to use them only on patients with a high predictability for benefit has been increased. The importance of understanding the different tumors that we might be facing is to be able to offer patients active therapies that will improve their survival mostly when the level of toxicity is high, and in the process be able to design studies in which we can enrich with populations that will increase the statistical power of the study with less patients, being able to advance faster the development of new therapies.

More importantly is to continue doing research on biomarkers so that we can better enrich and stratify clinical trials, and also to revisit even old trials in the search for potential biomarkers to be investigated in a prospective fashion. One of the main areas of discussion is that if we know so much about these markers, how come we can't better select patients for the studies? There are multiple explanations for this, including escape mechanisms of the tumor (resistance), regimen failure by itself or from lack of tolerability to pharmacologic interactions, and more importantly a biomarker failure that could be from many sources, for instance we might be looking at the wrong biomarker (as in the case of EGFR expression instead of KRAS mutation). Other reasons involve performing the wrong assay on the marker, the wrong

tissue being evaluated (as seen with VEGF where we have been focusing on the tumor instead of the stroma) and tissue acquisition in which at the moment the tissue is taken from the patient gene expression can change requiring fresh samples instead of paraffin embedded samples.

Recent studies that sequenced the genomes of breast and colorectal cancers have shown us that the mutations actually driving tumor progression are large in number and interspersed in a vast genomic landscape, confirming the tumor heterogeneity discussed earlier, but it was also found they likely function through common pathways which are already known to us. This discovery makes us one step closer to individualized medicine.[8]

An example of the advances in the field can be seen in breast cancer, where of late a specific tumor is not only being referred to as its tissue of origin, such as ductal or lobular, but we have encountered in the literature that behavior can be predicted based on the genetic expression which divides tumors in six different types.

It is not unthinkable that in the near future besides just dividing tumors by their organ of origin and then proceeding with staging, tumors will first be classified based on their genetic and antigenic markers and then decided whether a traditional 4-stage system will even be needed, since tumors with very good prognosis might behave equally after a curative resection despite being a traditional stage I or III. By the same token it is also within reach that treatments will be designed specifically for

each patient based on their metabolism and tumor biology, allowing for truly personalized medicine.

References

1. Ichikawa W, Takahashi T, Suto K, Yamashita T, Nihei Z, Shirota Y, et al. Thymidylate synthase predictive power is overcome by irinotecan combination therapy with S-1 for gastric cancer. Br J Cancer 2004; 91:1245-50; PMID:15354215; http://dx.doi.org/10.1038/sj.bjc.6602139.

2. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003; 349:247-57; PMID:12867608; http://dx.doi.org/10.1056/NEJMoa022289.

3. Hutchins G, Southward K, Handley K, Magill L, Beaumont C, Stahlschmidt J, et al. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. J Clin Oncol 2011; 29:1261-70; PMID:21383284; http://dx.doi.org/10.1200/JCO.2010.30.1366.

4. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008; 26:1626-34; PMID:18316791; http://dx.doi.org/10.1200/JCO.2007.14.7116.

5. European consortium data. Lancet Oncol 2010; 11:753-62; PMID:20619739.

6. Loupakis F, Pollina L, Stasi I, Ruzzo A, Scartozzi M, Santini D, et al. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. J Clin Oncol 2009; 27:2622-9; PMID:19398573; http://dx.doi.org/10.1200/JCO.2008.20.2796.

7. Siena S, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. J Natl Cancer Inst 2009; 101:1308-24; PMID:19738166; http://dx.doi.org/10.1093/jnci/djp280.

8. Wood LD, Parsons DW, Jones S, Lin J, Sjöblom T, Leary RJ, et al. The genomic landscapes of human breast and colorectal cancers. Science 2007; 318:1108-13; PMID:17932254; http://dx.doi.org/10.1126/science.1145720.