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Differences in Mitotic Activity May Explain Observed Differences in HIF-1 α Response in Cancers and Stroma

Khush Mittal

I read the paper by Chi et al. with interest. The authors found a greater hypoxia response gene expression in carcinomas than stromal cells grown in vitro, but were not sure of the underlying explanation (see Discussion in [1]). The underlying explanation may be the difference in mitotic activity and hence metabolic activity between these two cell types, such that hypoxia may be present within cancer cells, although the surrounding culture media may have similar oxygen levels as the stromal cultures.

The differences in mitotic activity could also explain greater hypoxia-related gene expression in clear-cell carcinoma compared with chromophobe carcinoma, normal tissue, or oncocytoma, as clear-cell carcinomas are more active mitotically. Did the authors compare the hypoxia gene expression with mitotic activity in various tumors? ■

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Borderline Thrombocytopenia or Mild Idiopathic Thrombocytopenic Purpura?

Jacques Zimmer, François Hentges, Emmanuel Andres

We read with interest the paper by Stasi et al. [1] about the follow-up of patients with a so-called borderline thrombocytopenia with platelet numbers of $100 \times 10^9/l$ to $150 \times 10^9/l$. Data about the long-term outcome of such patients are indeed not frequent in the literature, as the authors claim, but some information has nevertheless previously been published, as in our retrospective study on adult idiopathic thrombocytopenic purpura (ITP) [2], not cited by Stasi et al. [1].

Our cohort was composed of 201 ITP patients separated by treated and untreated individuals. The latter group was composed of 62 patients (30.8% of the cohort) with a women/men sex ratio of 3.1/1 and a mean age of 39 years.

The vast majority (54 patients, 87.1% of the untreated) were referred to the hospital because of mild isolated thrombocytopenia discovered on routine laboratory examination. These patients were asymptomatic and considered apparently healthy. In eight other cases (12.9%), however, a single, moderate, and spontaneously regressive bleeding episode occurred: purpura ($n = 4$), epistaxis ($n = 1$), gingivorrhagia after tooth extractions ($n = 1$), and meno- and metrorrhagia ($n = 2$) in two women with an intrauterine device and uterine polyps, respectively. The mean platelet count among these patients was $62 \times 10^9/l$, compared with $88 \times 10^9/l$ for the entire untreated group. Antinuclear antibodies were tested in 46 cases (74.2%), with significantly positive values in six patients (13% of the tested individuals). During the follow-up period of 1.9 to 59 months, no further bleeding occurred in the eight patients with initial moderate hemorrhage, although six of them remained thrombocytopenic for more than six months. None of the asymptomatic individuals developed any hemorrhagic symptoms. A chronic ITP (isolated thrombocytopenia of at least six months' duration) was diagnosed in 31 patients (59%) with a mean platelet count of $66 \times 10^9/l$. No autoimmune or hematological disease (other than ITP) developed in this group. Twenty-three patients were readdressed to their family physicians for further surveillance and lost to follow-up.

According to current guidelines for the management of adult ITP [3,4], patients with platelet numbers $>50 \times 10^9/l$ are usually not treated. The stable and moderate evolution of the thrombocytopenia in our patients confirms the validity of this approach. The fact that we did not observe the development of other autoimmune diseases in contrast to the small number of such cases noticed by Stasi et al. [1] might be related to the smaller size of our cohort and/or the shorter follow-up period. As these authors define ITP as a persistent platelet count of less than $100 \times 10^9/l$ and not $150 \times 10^9/l$ as we did, a direct comparison of the results might be difficult. Whether a patient with platelet counts of less than $150 \times 10^9/l$ but more than $100 \times 10^9/l$ is considered to have ITP or borderline thrombocytopenia is an academic question rather than a point of clinical importance, because the medical strategy is exactly the same (surveillance of platelet counts in the absence of any treatment). This holds true for patients with platelets between $50 \times 10^9/l$ and $100 \times 10^9/l$ that would be classified as ITP cases according to Stasi et al. [1]. Thus, it is not obvious why an additional clinical entity (borderline thrombocytopenia) should be created in addition to ITP. ■

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Authors' Response to Zimmer et al.

We wish to thank Dr. Zimmer and colleagues [1] for critically reviewing our paper [2] and bringing to our knowledge their results. We partly agree with their comments, especially the part when they affirm that a direct comparison of the results is difficult.

First of all, the design of the two investigations was different: ours was prospective and theirs was retrospective. Secondly, their data can hardly be interpreted and so cannot be a matter of contention. In fact, they merely report a mean platelet count of $88 \times 10^9/l$ for the untreated group of 62 idiopathic thrombocytopenic purpura patients and of $66 \times 10^9/l$ for the 31 patients later reclassified as having chronic idiopathic thrombocytopenic purpura. More importantly, they do not specify the number of their patients with a platelet count between $100 \times 10^9/l$ and $150 \times 10^9/l$, i.e., the class of individuals that was the focus of our study.

As an additional confounding factor, they report a follow-up period of 1.9 to 59 months for the entire untreated group. If a median is not reported, this does not make much sense statistically. Theoretically, 31 patients might have been followed for 1.9 months, 30 patients for six months, and one single patient for 59 months. If this was the case, no wonder they did not observe a single case of autoimmune disease in their cohort. We do not share Dr. Zimmer's point about a platelet count of $51 \times 10^9/l$ to $100 \times 10^9/l$ as equivalent to a higher count. Subjects who have a platelet count in the range of $50 \times 10^9/l$ to $80 \times 10^9/l$ are limited in their performance of particular physical jobs or traumatic activities such as contact sports. Besides, current guidelines suggest that a "safe" platelet count for major surgery, cesarean section, and spinal or epidural anesthesia should be at least $80 \times 10^9/l$ [3]. Therefore, these patients may occasionally require an evaluation and possibly treatment that is not required for those with a borderline thrombocytopenia.

Finally, we definitely rebut the issue of creating an unneeded clinical entity. The goal of our study was simply to describe the long-term outcome of individuals who were incidentally found with a platelet count between $101 \times 10^9/l$ and $150 \times 10^9/l$. The terms "borderline thrombocytopenia" should be interpreted only as the definition of a count in that range, not as a new clinical entity. In fact, we have clearly underlined that the majority of individuals will retain their borderline platelet count indefinitely without

developing diseases. Only a prospective case-control study would establish whether such individuals have a higher risk of developing autoimmune disorders than the general population. Until then, these cases should be interpreted only as healthy individuals with a platelet count in the lower range of normal. ■

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Schizophrenia Adoption Studies

Jonathan Leo

Patrick Sullivan cites twin and adoption studies as justification for searching for schizophrenia genes [1]. In his words, "Both adoption and twin studies indicate that the familiarity of schizophrenia is due mainly to genetic effects." To support this he provides a table (Table 1 in [1]) briefly summarizing these studies. Under "Adoption," he mentions, "Adoptees with schizophrenia: increased risk in biological vs. adoptive parents (OR = 5.0; 95% CI 2.4–10.4)." However, rather than making a comparison between biological and adoptive parents, the original investigators made comparisons between index biological relatives and control biological relatives.

In the schizophrenia literature three studies have used this design; the lead author of all three was Seymour Kety (1968, 1975, and 1994). All three studied people who grew up as adopted children and were later diagnosed with a "schizophrenia spectrum disorder." The goal was to examine the rate of schizophrenia spectrum disorders among those

adoptees' biological family members (with whom they did not grow up) and compare that rate to the rate among the biological relatives of control adoptees, who were not diagnosed with a schizophrenia spectrum disorder [2].

According to Kety himself, a comparison between index adoptees' biological and adoptive relatives is "improper" and "fallacious" [3,4]. Indeed, in Kety's first adoption study (1968) there was no significant elevation of chronic schizophrenia, or of schizophrenia spectrum disorders, among his index biological versus index adoptive relatives. (This is based on the data; the investigators did not make this comparison.)

In addition, Kety and his colleagues did not limit themselves to looking at only parents. If they had done so, their conclusions would have been very different. For instance, in the 1975 study there were five index biological relatives diagnosed with chronic schizophrenia, but four of these five were half-siblings. The other diagnosis was given to a biological parent. In the 1968 Kety study, not a single index biological parent was diagnosed with chronic schizophrenia.

In his conclusion, Sullivan says that the treatment of the mentally ill mirrors the humanity of a society. True, but it then becomes difficult to rationalize the treatment of schizophrenia in this country in light of the World Health Organization studies showing that doctors in developing countries use less medication yet have a higher success rate than doctors in America. This is well documented in Robert Whitaker's book *Mad in America: Bad Medicine, Bad Science, and Enduring Mistreatment of the Mentally Ill* [5]. ■

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Author's Response to Dr. Leo

Jonathan Leo raises issues with the adoption literature on schizophrenia [1]. These studies were intensively and independently scrutinized in the 1980s—see the series of papers by Kendler and Gruenberg (e.g., [2]). Most would agree that the number, size, and quality of adoption studies do not provide the highest-quality data (as discussed at more length elsewhere [3]).

However, the salient point in my paper [4] was that this body of work (twin, adoption, and family studies) provides

a consistent and solid rationale for the search for genes for schizophrenia.

Dr. Leo's comments about the treatment of schizophrenia are not within the scope of my paper. ■

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Telemedicine Complements Effective Health-Care Delivery but Is Not a Panacea

Rajiv Sarin

Like most health researchers in India, I share Sanjit Bagchi's enthusiasm and optimism about the potential of telemedicine in improving health-care delivery in our diverse country [1]. However, it is important to maintain a balanced perspective and debunk overstatements such as those used by Bagchi in his conclusion "This potential was well summed up by Dr. Devi Shetty: 'In terms of disease management, there is [a] 99% possibility that the person who is unwell does not require [an] operation. If you don't operate you don't need to touch the patient. And if you don't need to touch the patient, you don't need to be there. You can be anywhere, since the decision on healthcare management is based on history and interpretation of images and chemistry ... so technically speaking, 99% of health-care problems can be managed by the doctors staying at a remote place—linked by telemedicine.'"

Maybe Devi Shetty, well known in telemedicine circles, has been quoted out of context, but it is not evident as such. In the wider context of the story, such conclusions as "If you don't operate you don't need to touch the patient"; "And if you don't need to touch the patient, you don't need to be there"; and "so technically speaking, 99% of health-care problems can be managed by the doctors staying at a remote place" are misleading. In the near future, online physicians or health professionals cannot replace the onsite ones for even 30% of all health problems, let alone 99% as pointed out by Shetty. Even for providing essential and readily accessible health services, we need to augment the number of onsite

physicians and other health professionals with improved skill-mix, more pragmatic public health policies, and enabling of telemedicine. Though India has been a pioneer in evaluating telemedicine for improving health-care delivery [2,3], its rightful place can be established only through major coordinated research efforts [4].

Free-access medical journals are a forum for debate on emerging health issues and have the responsibility to implement rigorous peer review and editorial control to prevent wide dissemination of any misleading statements or misquotes, as the case may be. Sweeping concluding statements from opinion leaders appearing in respectable journals may unduly influence those lacking insight in the challenges of health-care delivery in underserved regions and allow further propagation by medical journalists living in virtual reality.

To realize its full potential, we need to identify more innovative applications of telemedicine and generate robust data on its cost-effectiveness in different health-care settings [4]. But first we need changes in skill-mix [5], which includes enhancement of skills among various staff groups, role substitution between different groups, delegation up and down a uni-disciplinary ladder, and innovation in roles. Until we have telemedicine models that are universally implementable, with proven cost-effectiveness and user satisfaction [6], we need a stance of cautious optimism. ■

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Further Advantages of a Unique Author Identification Number

Etienne Joly

I am in complete agreement with the suggestion by Matthew Falagas [1] that a unique author identification number (UAIN) would represent a major improvement for the

use of databases of scientific publications. In this regard, I perceive that he has not mentioned several other important advantages that a UAIN system would provide, and that are worth pointing to:

1. When looking up someone's publications, the fact that the last name of a given person can vary from one paper to another can be as much of a problem as that of multiple authors with the same name. For example, these variations include women who change their last name after getting married (or divorced), middle initials that are sometimes included or omitted, translations from non-Roman alphabets that result in variable spellings, and people with last names composed of several terms that can sometimes appear in databases as split or truncated.

2. Contrarily to M. Falagas, I do not see any good reason why a UAIN system could not be retroactive. It is clearly in every scientist's interest to facilitate the job of other people who want to look up their work. I therefore believe that authors could be asked to register for a UAIN, and to validate their list of publications themselves, retroactively. Even for the most productive scientists, this would take only a few minutes, and the fact that they had registered for a UAIN allowing users to trace their whole list of publications could then be indicated in the display of search results from the various bibliographic databases. I also do not see any reason for "hiding" this UAIN. I suggest that it could be designed to be quite simple to remember and to communicate to others, for example: the first four or five letters of the last name followed by the initial of the first name followed by the year of first scientific publication followed by an incremental number depending on order of registration (my UAIN would be JOLYE-89-01). It would therefore be something quite comparable in length and spirit to a car's licence plate and, like UK licence plates, it would provide an interesting clue regarding the seniority of its bearer.

3. This type of UAIN would therefore provide a very simple way to assess a person's productivity. It would also provide a very useful means to assess the actual impact of their work in terms of citations, by discriminating between self-citation and citations by others.

Today, most people are evaluated via the impact factor of the journals in which they have managed to publish their work, and not by the actual impact of the papers themselves. Although most scientists acknowledge that this is an extremely crude and unfair way of assessing the quality of someone's production, the impact factor lives on. By providing the simple means to track someone's bibliographic record and thus facilitate the evaluation of their productivity, I believe that the introduction of a UAIN system will not only help the scientific community to exploit bibliographic databases more efficiently, but also represent a major step towards getting rid of the despotic domination of the dreaded impact factors of journals as a means to evaluate the quality of scientific papers. ■

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Discussion Required for Correct Interpretation

Radek Bukowski, Gary D. V. Hankins, George R. Saade, Steve Thornton

Thank you for the opportunity to comment on the editorial by Romero and colleagues [1], which raises a number of important and interesting questions. Such discussion is mandatory if results of scientific techniques such as gene array are to be correctly interpreted and used as the basis for future improvements in patient care.

It is interesting that re-analysis of our results does not demonstrate significant changes in gene expression and highlights the importance of the analysis technique in data interpretation. We agree with the editorial that there is a wealth of data that supports labour-associated changes in gene expression [2–5] and any implication that there are no significant labour-associated differences would ignore the results of numerous targeted analyses published by many independent researchers worldwide. Indeed the number and quality of such publications led us to make the basic assumption that there are labour-associated changes in gene expression. For this reason we calculated the *p*-value to identify those genes with the greatest difference in expression and smallest variability rather than to determine significance. This analytical design was strengthened by gene array analysis of each patient sample rather than analysis of pooled mRNA. We accept that there are a number of techniques available that can determine statistical significance whilst attempting to account for multiple analyses in gene array studies. However, those genes most likely to be important for the biological process of labour may or may not demonstrate a significant change in expression when corrections are made for multiple analyses. Furthermore, we have demonstrated that changes in the expression of individual genes are not independent, which complicates analysis. For example, the false discovery rate assumes a certain level of correlation among genes. However, the size of this correlation is not known and if underestimated can result in a high false negative rate. Thus, in keeping with other human uterine gene array data [6,7], we believe that there are likely to be important biological changes in labour-associated gene expression that may or may not be reflected by finding statistical significance of a test chosen for analysis. We agree that the preparation (activation) of the uterus for the onset of labour is likely to occur in the weeks preceding its onset and such changes cannot be expected to be identified by labour and non-labour comparisons of gene expression.

Our original analysis included patient data, which was removed during re-analysis by Romero et al. Data from

one patient were omitted because the arrays demonstrated saturation. A second set of data was removed because the modal probe intensity was 8-fold higher than the others. We normalised our data by transforming the expression value into a percentile and giving this as a multiple of the standard deviation (*z*-score). Thus, in contrast to some other methods of transformation (e.g., logarithmic), ours is insensitive to the magnitude of expression since genes are given a rank relative to other genes. Data from a third patient were removed because a different Affymetrix chip was used. We considered that inclusion of data from this chip was appropriate since it is almost identical to the original (all apart from 25 of the 12,626 genes), and ranking genes by their *z*-scores removes chip-to-chip differences.

Our analysis identified genes that demonstrate a marked labour-associated difference in expression. We used this data to identify networks of genes that are co-regulated. We believe that the process of labour does not result from the independent expression of single genes but the effect of coordinated regulation of groups of genes that act in synchrony on a primed uterus to initiate labour. ■

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