Author’s response to reviews

Title: NF-kappa B genes have a major role in Inflammatory Breast Cancer

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Author’s response to reviews:

Please find enclosed our revised manuscript ¿NF-KAPPA B GENES HAVE A MAJOR ROLE in INFLAMMATORY BREAST CANCER¿ by Lerebours et al. The manuscript has been revised in view of the reviewers¿ comments.

You will find a point-by-point answer to these comments.

We hope you will find the revised form suited for publication in BMC Cancer, and look forward to hearing from you.

Sincerely yours,

F. Lerebours, MD PhD

Reviewer: Muzaffer Cicek

This study was performed on small tumor biopsies mainly collected between 1988 and 1991. Priority was given to the diagnosis on formalin-fixed samples. A small piece of the biopsy was frozen for molecular analysis. To date, there are no more paraffin-embedded samples available for IHC analysis corresponding to these biopsies.

We are planning IHC studies on recently diagnosed IBC samples that we have already started to collect at our institution. We aim to prove whether NFkB regulated genes, notably the 6 genes separating IBC from non IBC, are involved at the protein level in IBC.

Huge ranges of genes’ expression at the mRNA level are not unusual in breast tumors. For instance it has been observed for the oestrogen receptor ERA and the tyrosine kinase receptor ERBB2. In IBC, it has also been reported for several genes in other RT-PCR studies (in the study by van Laere et al., CD48 mRNA level ranged from 0,1 to 32 in IBC samples; van Laere et al, Breast Cancer Res
Numerous genes with a low baseline mRNA expression level show high upregulation in tumor samples specially when studied using real-time quantitative RT-PCR. Finally, it should be born in mind that several genes tested in the present study are cytokines or chemokines that are known to be expressed at very various levels in tumors.

As the mRNA levels of the studied genes did not fit a Gaussian distribution, the relationships between their expression levels and clinical data were tested with a non-parametric test. The significance of gene’s upregulation is thus based on its median mRNA level rather than the mean level in a group of tumors compared with another group. It accounts for less striking differences between two groups. The significance of results is assessed by the p value with a conventional threshold of 0.05. For example, the median expression level of MCL1L in IBC samples is only 2 fold higher than in non IBC samples. However, this upregulation is highly significant (p value = 0.000035).

Reviewer: Nathalie Cervera

Major comments

This is a major point. However, only few research groups have published data on DNA microarrays in IBC and only one of these studies provides accessible gene expression and histoclinical data (Bertucci F et al, Cancer Research, 64: 8558-65, 2004). The 6 genes of the signature differentiating our series of IBC from non IBC are present on the cDNA microarrays used in that study. The 6-gene signature was thus tested on this independent set of 37 IBC and 44 non IBC samples. Based upon a filter procedure eliminating non informative genes (expression levels < 2 X background signal in at least 50% of all samples), two of the 6 genes CCND3 and SELE- were retained. Hierarchical clustering of the 37 IBC and 44 non IBC samples based on these 2-gene expression defined two classes significantly correlated with the tumor phenotype (p=0.01). The study by Bertucci et al did not provide information on patient prognosis, so that our prognostic 5-gene signature could not be tested on this independent set of IBC tumors.

The choice of associating CCND3 with the group RELA/IKBKG/NFKBIB is debatable. In other words, CCND3 may have been associated with the group including CXCL12. However, we chose to separate significantly dysregulated genes in small groups (< 7-8 genes).

Minor comments

The induction of GADD45B by stress and cytokines has been shown to require NFKB genes (Karin and Lin, Nature, 3:221-227, 2002). Likewise, NFKB genes upregulate VEGF and Cyclins expression levels. The term NFKB pathway genes can thus be extended to such genes. Finally, these genes are often mentioned as NFKB pathway genes in several reviews (Karin et al, Nature Rev, 2:301-301, 2002).