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Stem cell mobilisation by hyperbaric oxygen (HBO)

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Background: A recent article reported that exposure to hyperbaric oxygen (HBO) causes rapid mobilization of endothelial stem cells (SC) in humans [1]. The population of CD34+ cells in the peripheral circulation of humans doubled in response to a single exposure to 2.0 atmospheres absolute (ATA) O2 for 2 hours, and increased 8-fold over a course of 20 treatments. Since these results offer new insights into a possible mechanism of action of HBO therapy in radiation injuries, we tested the levels of CD34+ cells in the peripheral blood of patients undergoing 30 sessions of HBO therapy (each at 2.4ATA for 90 min) for radiation-induced arm lymphoedema and attempted phenotypic characterisation of the induced SC.
Material and Methods: Blood samples were collected before & after the 1st session of HBO & after the 16th & 26th sessions. We also tested for an association between age and/or previous radiotherapy & SC induction to a single session of HBO in a sample of healthy human volunteers (<40 yrs or >60 yrs). Flow cytometry using a panel of markers shown in the Table was used to identify & quantify haematopoietic & mesenchymal SC.

Fluorochrome	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5
FITC	CD45	CD45	CD45	CD45	CD45
PE	CONTROL	CXCR4	CD71	CD33	CD135
PER-CP CY5.5	CD34	CD34	CD34	CD34	CD34
APC	CONTROL	VEGFR2	CD38	CD133	CD117

Results: In the 1st five evaluable patients, the baseline levels of CD34+ cells ranged from 0.020 to 0.049% of 100,000 events with a mean of 0.0326% which is consistent with published literature [2]. After 26 sessions of HBO the mean CD34+ count was 0.0388% (range 0.022−0.057%), an average increase of 1.2 fold (95%CI: 0.8−1.6, p = ns). The CD34+/VEGFR2+ subtype showed a baseline mean of 0.001% (range 0.000−0.002) with a rise to 0.007% (range 0.000−0.014%) at the end of treatment. Further endothelial characterisation by CD45− subgroup analysis is currently underway. The 5 normal volunteers <40 yrs had a mean baseline CD34+ count of 0.0276% with a level of 0.031% after 1 HBO session. Six volunteers >60 yrs showed a non-significant change in mean CD34+ levels from 0.0355% to 0.0303% after 1 HBO session.
Conclusions: So far, we have not been able to detect the 8-fold increase in CD34+ cells in response to HBO reported by Thom et al. Further research is needed to confirm or refute this potentially important therapeutic mechanism.

References

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Expression of hypoxia-related genes in papillary thyroid cancer

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Purpose: Hypoxia is a well known feature of malignant tumors, decreasing their response to radiation therapy. Until now it has not been widely studied in thyroid cancer. In the present study we analyzed the changes in expression profile of genes found to respond to hypoxia in our previous microarray-based studies of malignancy. Among them were genes participating in angiogenesis (VEGF, ADM, SPARC), apoptosis (BNIP3, PBEF1, IER3, ANXA2, indirectly also MAP3K1), cell adhesion and migration (LGAL3, VCL, CTSB), glucose metabolism (GLUT1, HK2, ALDOC) and some others, in total 28 transcripts.
Material and Methods: The analysis was carried out in 31 papillary thyroid cancers (PTCs) taken intraoperatively and respective normal surrounding thyroid tissue (ST). QPCR was carried out by Universal Probe Library probes on ABI 7900HT. Gene expression values were normalized against three-gene index, selected form 10 house-keeping transcripts by GeNorm software.
Results: For 17/28-related gene the difference in gene expression between tumor and ST was significant, for 13/28 genes at $p < 0.001$. All of them but VEGF showed higher expression in PTC than in ST. The highest difference in expression (on average 8.2x) was seen for LGAL3 and KDELR3 (4.4x). The overexpression was particularly visible for hypoxia-related cell adhesion and migration genes, proline hydroxylases and lysosomal degradation related genes. Contrary to our expectations, VEGF gene showed no distinct up-regulation, but other pro-angiogenic factors like adrenomedullin (ADM) could exert similar effect. Genes controlling glycolysis were inconsistently up-regulated – the overexpression of HK2 was not accompanied by GLUT1 expression change.
Conclusions: Hypoxia-related cell adhesion and migration genes are up-regulated in PTC which contributes to its metastatic potential. Simultaneously, changes in apoptosis related genes indicate on its reduced apoptotic potential.
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Tumor markers differentiating between pancreatic cancer and normal pancreas/chronic pancreatitis: gene expression-profiling study

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Background: We applied multivariate methods of class prediction to derive novel molecular markers, differentiating between pancreatic cancer and normal pancreas/chronic pancreatitis, based on the results of gene expression profiling by oligonucleotide microarrays.
Materials and Methods: The snap-frozen or RNA-later preserved samples of 18 pancreatic adenocarcinomas, 9 chronic pancreatitis cases and 6 specimens collected from microscopically unchanged pancreas (N/CP) were analyzed by HG-U133 Plus 2.0 oligonucleotide microarrays (Affymetrix). The obtained dataset was pre-processed using GC-RMA method, gene selection was carried out both by class comparison methods (Welch test with Benjamini-Hochberg correction, False Discovery Rate FDR < 5%) and by our own algorithms of class prediction, based on Support Vector Machines technique (Recurrent Feature Replacement and Bootstrap-Based Feature Ranking). Real-time quantitative PCR (Q-PCR) was carried out on Applied Biosystems 7900 HT machine, with Universal Probe Library (Roche) fluorescent probes and normalization by three reference genes index (geNorm, Vandesompele et al.).
Results: Gene expression profiles of pancreatic cancer samples and N/CP specimens were compared. With False Discovery Rate threshold set to 5%, 23850 probesets significantly differentiated between these three classes. No ideal discrimination between cancer and N/CP samples was possible by any of single markers. We selected the optimal multi-gene classifier by Support Vector Machines, using Bootstrap-Based Feature Ranking method. The smallest classifier resulting in 100% accuracy consisted of three genes, 45 genes were included in more than half of the diagnostic genesets obtained during bootstrapping process. 14 genes were selected for Q-PCR validation, again none of them ideally discriminated between cancer and normal specimens, with the area under the receiver-operating-characteristic curve ranging from 0.82−0.93. Three-gene combinations allowed for proper classification of all samples.
Conclusions: Molecular multigene classifier is able to discriminate properly between pancreatic cancer and chronic pancreatitis/normal pancreas. At least three genes must be included in the classifier to obtain satisfying accuracy.
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