Letter to the Editor

p21 ras-related Protein Levels Depend on Sample Handling

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Proteins immunologically related to oncogene products are detectable in plasma, serum, and urine. We and others are studying the relationship between cancer risk and circulating proteins immunologically related to the *ras* (1–6), c-*erbB2* (7), c-*myc* (8), *int-1* (9), and other (10) oncogene products. We recently observed that p21 ras-related plasma protein levels depend on initial sample processing methods. Plasma from blood stored at room temperature for greater than 4 h showed an increase in p21 ras relative to samples processed immediately. The increase is likely due to release from cells, related proteins, or breakdown of larger molecules.

We carried out the following experiment with plasma from healthy volunteers to investigate the effect of sample handling on immunoassay of p21 ras-related proteins. Blood was drawn from eight healthy white subjects ranging in age from 23 to 52 years, 1 male and 7 female. Five-ml (lavender top) EDTA tubes were used. For each subject, the blood was divided into six aliquots and each one of the aliquots was subjected to each of the following conditions before analysis: (A) plasma immediately separated; (B) plasma immediately separated and kept at room temperature 4 h; (C) blood kept at ice temperature 4 h before separating plasma; (D) blood kept at ice temperature 8 h before separating plasma; (E) blood kept at room temperature 4 h before separating plasma; or (F) blood kept at room temperature 8 h before separating plasma.

We measured p21 ras-related plasma protein levels with an immunoblot assay technique previously described (1, 11–14). The monoclonal antibody used was 142.24E05. Fig. 1 shows p21 levels as integrated optical density or absorbance. Densitometry measurements were performed with the aid of a computer-automated system. Table 1 shows mean integrated absorbance values and SDs for conditions A–F.

Differences in median p21 ras-related plasma levels were statistically significant (Kruskal Wallis test; $P = 0.0001$). Two-sample (Wilcoxon) testing against control condition A showed increased median p21 ras-related plasma proteins from blood kept at room temperature for 4 h (condition E; $P = 0.001$) and 8 h (condition F; $P = 0.003$) before sample processing. Median p21 ras-related protein levels from blood processed according to conditions B, C, and D did not differ statistically from the control condition.

Table 1 Mean integrated absorbance of plasma p21 ras-related proteins

Conditions	Mean integrated absorbance	SD
A	0.0313	0.0179
B	0.0209	0.0143
C	0.0293	0.0171
D	0.0638	0.0461
E	0.143	0.0576
F	0.147	0.0768

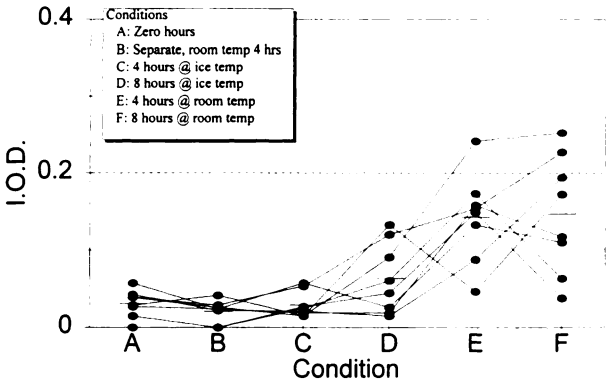

Fig. 1. Plasma p21 ras-related protein-integrated absorbance *versus* sample handling procedures for eight healthy volunteers. IOD, integrated optical density, or absorbance.

Therefore, we observed substantial effects of sample handling procedures on plasma levels of p21 ras-related proteins. These effects are clearly large enough to threaten the validity of epidemiological or clinical studies, particularly studies which entail separate sample collections from unique populations dispersed over time or place. We suspect that many other candidate cancer biomarkers may be susceptible to similar artifacts. Therefore, all reports pertaining to new cancer biomarkers should include a complete description of sample collection and storage procedures.

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